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Diagnostics Assessment Programme

Tests for rapidly identifying bloodstream bacteria and fungi (LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay)

Committee Papers

NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE

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Contents:

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Diagnostic Assessment Report commissioned by the NIHR HTA Programme on behalf of the National Institute for Health and Care Excellence

Title: Sepsis: The LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi. A systematic review and economic evaluation.

Produced by ScHARR, University of Sheffield

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Data can be obtained from the corresponding author subject to it being non-confidential

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Matt Stevenson (Professor of Health Technology Assessment) and Rachid Rafia (Research Fellow) were responsible for the acquisition of data, analysis and interpretation of data and model construction

(for the health economic evaluations) and drafting and revising of the final report. Abdullah Pandor (Senior Research Fellow), Marrissa Martyn-St James (Research Fellow) and Lesley Uttley (Research Fellow) co-ordinated the review and was responsible for the acquisition of data, analysis and interpretation of data (for the systematic review) and drafting and revising the final report. John Stevens (Reader in Decision Science) and Jean Sanderson (Research Associate) were responsible for the statistical analyses, interpretation of data and drafting and revising the final report. Ruth Wong (Information Specialist) was responsible for the developing and undertaking the electronic literature searches. Gavin D Perkins (Professor of Critical Care Medicine), Ronan McMullan (Senior Lecturer and Consultant Microbiologist) and Paul Dark (Reader and Honorary Consultant Intensivist) were responsible for providing expert clinical advice throughout the project and drafting and revising the final report.

About ScHARR

The School of Health and Related Research (ScHARR) is one of the nine departments that comprise the Faculty of Medicine, Dentistry and Health at the University of Sheffield. ScHARR specialises in health services and public health research, and the application of health economics and decision science to the development of health services and the improvement of public health.

The ScHARR Technology Assessment Group (ScHARR-TAG) synthesises research on the clinical effectiveness and cost-effectiveness of healthcare interventions for the NIHR Health Technology Assessment Programme on behalf of a range of policy makers, including the National Institute for Health and Care Excellence (NICE). ScHARR-TAG is part of a wider collaboration of a number of units from other regions including Health Economics Research Unit and Health Services Research Unit, University of Aberdeen; Southampton Health Technology Assessment Centre (SHTAC), University of Southampton; Liverpool Reviews & Implementation Group (LRiG), University of Liverpool; Peninsular Technology Assessment Group (PenTAG), University of Exeter; the NHS Centre for Reviews and Dissemination, University of York; Warwick Evidence, University of Warwick; the BMJ Technology Assessment Group (BMJ-TAG), BMJ Evidence Centre and Kleijnen Systematic Reviews Ltd.

ABSTRACT

Background

Sepsis can lead to multiple organ failure and death. Timely and appropriate treatment can reduce in-hospital mortality and morbidity.

Objectives

To determine the clinical effectiveness and cost effectiveness of three tests (LightCycler SeptiFast Test MGRADE (SeptiFast); SepsiTest; IRIDICA BAC BSI assay (IRIDICA)) for the rapid identification of bloodstream bacteria and fungi in patients with suspected sepsis compared with standard practice (blood culture with or without Matrix-absorbed laser desorption/ionization- time of flight mass spectrometry).

Methods

A systematic review and meta-analysis (where appropriate) of effectiveness studies was conducted. A review of published economic analyses was undertaken and a *de novo* health economic model was constructed. A decision tree was used to estimate the costs and quality-adjusted life years (QALYs) associated with each test. The model was populated with evidence from the systematic review or individual studies if this was considered more appropriate. Where evidence was lacking, estimates from expert clinicians involved in the management of patients with suspected sepsis was sought; all other parameters were estimated from published sources. An NHS and personal social services perspective was taken and costs and benefits were discounted at 3.5% per annum. Scenario analyses were used to assess uncertainty although deterministic results only were provided given the large variation between scenario results.

Results

For the review of diagnostic test accuracy, 62 studies of varying methodological quality were included. A meta-analysis of 54 studies comparing SeptiFast with blood culture found that SeptiFast had an estimated specificity of 0.86 (95% Credible Interval (CrI): 0.84 to 0.89) and sensitivity of 0.65 (95% CrI: 0.60 to 0.71). A meta-analysis of four studies comparing SepsiTest with blood culture found that SepsiTest had an estimated specificity of 0.86 (95% CrI: 0.78 to 0.92) and sensitivity of 0.48 (95% CrI: 0.21 to 0.74). A meta-analysis of four studies comparing IRIDICA with blood culture found that IRIDICA had an estimated specificity of 0.84 (95% CrI: 0.71 to 0.92) and sensitivity of 0.81 (95% CrI: 0.69 to 0.90). Due to the deficiencies in study quality for all interventions, diagnostic accuracy data should be treated with caution.

The economic analysis evaluated two key scenarios: using only statistically significant and non-confounded data from published literature (Base case 1) or using data provided by clinical experts (Base case 2). Base case 1 estimated that none of the three tests provided a benefit to patients

compared with standard practice and thus all tests were dominated. In contrast, in Base case 2 it was estimated that all cost per QALY gained values were below £20,000. IRIDICA had the highest estimated incremental net benefit but the results should be treated with caution.

Limitations

Robust data to accurately assess the clinical and cost effectiveness of the interventions are currently unavailable.

Conclusions

The clinical and cost effectiveness of the interventions cannot be reliably determined with the current evidence base. Appropriate studies, which allow information from the tests to be implemented in clinical practice, are required.

Study Registration

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Plain English Summary

Sepsis is estimated to cause 37,000 deaths per year in the UK. Early and appropriate treatment can reduce the risk of sepsis-related death. New tests can detect bacteria and fungi in the blood much quicker than standard practice allowing treatment changes to occur faster. The report looked at the clinical and cost effectiveness of three tests: The LightCycler SeptiFast Test MGRADE®; SepsiTest™; and IRIDICA BAC BSI assay. A review of the published literature showed that the tests were better at correctly identifying patients without sepsis than those with sepsis, and that all three tests were imperfect. However, due to limitations in reporting and study quality, these data should be treated with caution. A review of the published literature showed that the desired benefits (reduced mortality, reduced length of stay in intensive care units and in hospital, and in reduced costs of treatment) of the new tests had yet to be proven. However, expert clinicians were asked to provide estimates of these benefits and the answers were on average positive, although individual clinicians held widely different views. Given the markedly different results produced when the published evidence of benefits were used and when the estimates from the clinicians were used, no firm conclusions could be made regarding the likely cost or benefits associated with the three tests. In order to provide better estimates, studies should be undertaken where information from the tests is allowed to change clinical practice and for these results to be compared with those from current practice.

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DEFINITION OF TERMS AND LIST OF ABBREVIATIONS

Technical terms and abbreviations are used throughout this report. The meaning is usually clear from the context, but a glossary is provided for the non-specialist reader.

Term	Definition
Aliquot	A portion of a total amount of a solution
Amplicon	A piece of deoxyribonucleic acid or ribonucleic acid that is the source and/or product of natural or artificial amplification or replication events
Antigen	Any structural substance that serves as a target for the receptors of an adaptive immune response
Antimicrobial medications	including antibiotics, anti-fungals and anti-virals
Bacteremia	The presence of bacteria in the blood
Bloodstream infection	The presence of bacteria in the blood
Broad spectrum antibiotic	An antibiotic that acts against a wide range of disease-causing bacteria
CE mark	A manufacturer's declaration that the product meets the requirements of the applicable EC directives
Colony forming unit	A unit used to estimate the number of viable bacteria or fungal cells in a sample
Dominated	When an intervention is more expensive and provides equal or less additional quality adjusted life years or is equally expensive and provides less quality adjusted life years
Dominating	When an intervention is less expensive and provides equal or more quality adjusted life years or is equally expensive and provides more quality adjusted life years
Empiric treatment	A therapy based on clinical experience in the absence of complete information
Gram staining	Differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in gram-positive bacteria
Gram-indeterminate bacteria	Bacteria that do not respond predictably to Gram staining and, therefore, cannot be determined as either gram-positive or gram-negative
Gram-negative bacteria	Bacteria that give a negative result in the Gram stain test
Gram-positive bacteria	Bacteria that give a positive result in the Gram stain test
Incremental cost effectiveness ratio	The additional cost per unit increase in effectiveness (often measured in quality adjusted life years)
Incremental net monetary benefit	A measure of the cost effectiveness of a test at a given cost per quality adjusted life year gained threshold. The greater the incremental net monetary benefit the more cost effective the test
Index test	The test whose performance is being evaluated
Lysis	The breaking down of the membrane of a cell, often by viral, enzymic, or osmotic mechanisms that compromise its integrity
Maximum acceptable incremental cost effectiveness ratio	The largest value that society is assumed to be willing to spend to purchase one unit increase in effectiveness

Narrow spectrum antibiotic	An antibiotic effective against specific families of bacteria
Nosocomial infection	Hospital-acquired infection
Quality adjusted life years	A measure of both the longevity and quality of life. The higher the quality adjusted life years the longer a person is likely to live for and/or the better quality of life the person is predicted to have.
Polymerase chain reaction	A technology in molecular biology used to amplify a single copy or a few copies of a piece of deoxyribonucleic acid across several orders of magnitude, generating thousands to millions of copies of a particular deoxyribonucleic acid sequence
Propensity score matching	A statistical matching technique that attempts to estimate the effect of a treatment, policy, or other intervention by accounting for the covariates that predict receiving the treatment
Reference standard	The best test currently available
Sanger sequencing	A method of deoxyribonucleic acid sequencing
Sensitivity	The proportion of people with the target condition that receive a positive test result. It is not uncommon for the true status of the patient to be determined by the reference standard even if that is an imperfect test
Specificity	The proportion of people without the target condition, It is not uncommon for the true status of the patient to be determined by the reference standard even if that is an imperfect test.
Sepsis	A condition characterised by the body's inflammatory response to an infection
Septic shock	Persistent sepsis-induced hypotension (low blood pressure) despite adequate fluid resuscitation
Severe sepsis	Occurs when the body's response to infection interferes with the functioning of vital organs, such as the heart, kidneys, lungs or liver
Single gate	A study design in which only patients with the target condition are recruited
Superinfection	A second infection superimposed on an earlier one, especially by a different microbial agent of exogenous or endogenous origin, that is resistant to the treatment used against the first infection

Abbreviation	Term/Definition
APACHE	Acute physiology and chronic health evaluation
CI	Confidence Interval
CQUIN	Commissioning for quality and innovation payment framework
CRD	Centre for Reviews and Dissemination
CrI	Credible interval
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FN	False negative
FP	False positive
GDG	Guideline Development Group
HAP/HCAP	Healthcare-associated pneumonia
ICER	Incremental cost effectiveness ratio
ICU	Intensive Care Unit
MAICER	Maximum acceptable incremental cost effectiveness ratio
MALDI-TOF	Matrix-absorbed laser desorption/ionization- time of flight
MS	Mass spectrometry
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMB	Net monetary benefit
NR	Not reported
NS	Not significant
PCR	Polymerase chain reaction
PrI	Prediction interval
QALY	Quality adjusted life year
RCTs	Randomised controlled trials
SAPS	Simplified acute physiology score
sCAP	Severe community-acquired pneumonia
SIRS	Systemic inflammatory response syndrome
SIRS-SS	Systemic inflammatory response syndrome with suspected sepsis
SOFA	Sequential organ failure assessment
SROC	Summary receiver operating curve
TN	True negative
TP	True positive
USA	United States of America
VAP	Ventilator-associated pneumonia
VAT	Value Added Tax
WWW	World Wide Web

SCIENTIFIC SUMMARY

Background

Sepsis is a condition characterised by the body's inflammatory response to a bacterial, viral or fungal infection. In the UK sepsis is estimated to be responsible for 100,000 hospital admissions and 37,000 deaths per year. As a consequence, the cost to the NHS is considerable. Current standard practice for detecting pathogens in those with suspected blood stream infection or sepsis consists of clinical assessment in conjunction with blood culture (with or without Matrix-absorbed laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF MS)). However, positive blood culture results for bacteria or fungi can take take several days. Several new tests have been developed which can detect minute amounts of pathogen DNA in patients' whole blood samples with results available within approximately six hours under optimal conditions.

Objectives

To evaluate the clinical and cost effectiveness of three interventions (the LightCycler SeptiFast Test MGRADE,® (SeptiFast) SepsiTest™ and IRIDICA BAC BSI assay (IRIDICA)) for the rapid identification of blood stream bacteria and fungi compared with standard practice.

Methods

Clinical evidence review

A systematic review was conducted in accordance with established guidelines. Thirteen electronic databases and research registers were searched (including MEDLINE, EMBASE and the Cochrane Library) to May 2015. Searches were supplemented by hand searching of relevant articles (including citation searching and screening company submissions) and contacting experts in the field. The methodological quality of each included study was performed using the Quality Assessment of Diagnostic Accuracy Studies tool. Results were summarised in tables and text. Data analysis comprised a narrative synthesis and pair-wise meta-analysis.

Cost-effectiveness assessment

A systematic review of evidence relating to the cost effectiveness of the interventions was undertaken. A mathematical model was constructed with two key scenarios evaluated: Base case 1 where only published statistically significant data were used within the model and Base case 2 where data provided by clinical experts were used. Further analyses were conducted where studies had compared interventions where MALDI-TOF MS was used in conjunction with blood culture and where studies had compared two of the interventions simultaneously. Evaluations were undertaken assuming a range of blood samples that need analysing per day (2.4 to 68) for all scenarios. Threshold analyses were also undertaken to provide further information for the Diagnostic Appraisal Committee on the gains

required in reduced mortality, reduced intensive care unit length of stay and in reduced costs of antimicrobials.

Results

Clinical effectiveness results

The literature searches identified 2892 citations. Of these, 66 studies met the inclusion criteria. The methodological quality of the included studies was variable, with the majority having deficiencies in reporting and study quality. For the review of diagnostic test accuracy, a meta-analysis of 54 studies comparing SeptiFast with blood culture found that SeptiFast had an estimated specificity of 0.86 (95% Credible Interval (CrI): 0.84 to 0.89) and sensitivity of 0.65 (95% CrI: 0.60 to 0.71). However, there was substantial heterogeneity between studies, particularly for sensitivity. Reasons for the observed heterogeneity in sensitivity and specificity between studies were explored using meta-regression for several potentially relevant characteristics: age category (adults and children and neonates), antibiotic use at the time of blood sampling, community or health acquired infection, patients with febrile neutropenia and studies with inclusion/exclusion of contaminants. There was no evidence to suggest that the pooled sensitivity and specificity was affected by these subgroups. Comparison with blood culture plus MALDI-TOF MS in a single study showed higher specificities than sensitivity (0.74, 95% CrI: 0.64 to 0.85 and 0.58, 95% CI: 0.30 to 0.86, respectively). Pooled effects across four studies comparing SepsiTest with blood culture suggest that SepsiTest had an estimated specificity of 0.86 (95% CrI: 0.78 to 0.92) and sensitivity of 0.48 (95% CrI: 0.21 to 0.74). Although, there was substantial heterogeneity between studies, analyses for potential causes of this heterogeneity could not be explored due to the small number of included studies. Comparison with blood culture plus MALDI-TOF MS in a single study also showed higher specificities than sensitivity (0.96, 95% CrI: 0.92 to 1.00 and 0.11, 95% CrI: 0.00 to 0.23, respectively). A meta-analysis of four studies comparing IRIDICA with blood culture found that IRIDICA had an estimated specificity of 0.84 (95% CrI: 0.71 to 0.92) and sensitivity of 0.81 (95% CrI: 0.69 to 0.90). However, there was substantial heterogeneity between studies. Moreover, due to the deficiencies in study quality for all interventions diagnostic accuracy data may not be reliable and should be treated with caution.

For the review of other intermediary and clinical outcome measures, 41 studies across the three interventions reported data on time to pathogen identification (SeptiFast only, n=21); time to treatment (SeptiFast only, n=3); test failure rates (SeptiFast, n=7 [redacted]); duration of stay in hospital or critical care units (SeptiFast only, n=13); duration of broad and narrow spectrum antimicrobial therapy (SeptiFast only, n=1); changes in antimicrobial treatment plan (SeptiFast, n=14 [redacted]) and mortality (SeptiFast, n=17; SepsiTest, n=1; [redacted] and SeptiFast/SepsiTest, n=1) only. The majority of studies reported data for the whole patient cohort, as opposed to comparative data for the index and reference test. As a result, the effects of the individual test on these outcomes remains unclear.

Cost-effectiveness results

Four economic evaluations were identified, three evaluating SeptiFast and one evaluating a hybrid of IRIDICA and an earlier system PLEX-ID, but none were deemed to adequately address the decision problem. The results produced by the *de novo* model were highly variable. In Base case 1 all interventions were dominated as the tests were not assumed to provide benefit. In Base case 2 all interventions were estimated to have cost per quality adjusted life year (QALY) gained values of less than £20,000 when using the average values provided by the clinicians; however, these estimates differed markedly between individual clinicians with a non-negligible proportion believing the tests had a cost per QALY gained in excess of £30,000. IRIDICA was estimated to have the greatest net monetary benefit, followed by SepsiTest and then SeptiFast. The additional analyses undertaken using the results from multi-test studies that compared SeptiFast, SepsiTest and blood culture, when the data provided by clinicians were used, were concordant with Base case 2. However, the indirect results produced when using studies directly comparing to MALDI-TOF MS produced contrary results with SeptiFast estimated to dominate SepsiTest. Within the threshold analyses it was seen that relatively small mortality gains would be required for the interventions to achieve a cost per QALY gained of £20,000 compared with standard practice.

Discussion

SeptiFast, SepsiTest and IRIDICA appear to have higher specificity values than sensitivity values. However, given the potentially fatal consequences of removing treatment from patients with sepsis it is not anticipated that negative tests in isolation would be acted upon in clinical practice were an intervention introduced. Moreover, due to the deficiencies in study design and poor reporting of the included studies, these data may not be reliable and should be treated with caution.

The pooled estimates of sensitivity and specificity for each test were estimated assuming that the reference standard was 100% sensitive and specific; however, this is unlikely to be the case. In practice, a wide range of factors are known to influence the diagnostic accuracy of blood cultures. For example this may include antimicrobial treatment prior to blood sampling, low blood sample volumes, lack of replicate blood culture sets, delays in incubation and contamination during sampling. As a result, the reported estimates of sensitivity and specificity are likely to be biased (underestimated) compared to those that would be obtained using a perfect reference standard. In addition, diagnostic metrics in the included studies were measured using different units: patients, sample episodes or species/pathogen level. Such analyses create a 'unit of analyses' error and may have contributed to the heterogeneity in the results.

Although there are no existing systematic reviews of diagnostic accuracy for SepsiTest or IRIDICA, the present review includes more studies than previous reviews on SeptiFast and is therefore more

comprehensive. Although an extensive literature search was conducted, it is possible that some studies may have been missed. However, such omissions are likely to have been minimal as the search included all identifiable publications in the grey literature (including contact with clinical experts in the field and checking evidence submitted by the companies that manufacture the tests). Statistical evaluation of diagnostic test accuracy was undertaken using rigorous methods, allowing for the correlation between sensitivity and specificity, and between study heterogeneity. Reasons for the heterogeneity in sensitivity and specificity between studies were further explored using meta-regression. Parameter estimates were produced using Markov Chain Monte Carlo simulation.

There are no head to head comparisons of all these tests and there are limited, robust data that report the impact of interventions on hard clinical outcomes such as mortality and reduced length of stay in critical care units. The data that do exist have not shown any intervention to produce a non-confounded statistically significant improvement. In addition, the three interventions provide very limited data regarding antimicrobial sensitivity. In addition, the three interventions provide very limited data regarding antimicrobial sensitivity. Definitive data on this is needed to be determined, if possible, via standard culture methods undertaken in parallel with the interventions. In order to produce a definitive conclusion on the clinical effectiveness of these interventions, appropriate studies need to be conducted.

The results from the cost effective analyses are fundamentally limited by the lack of appropriate evidence. As such, little credence should be given to any result. However, the results from Base case 2 show that there appears to be clinical support for the effectiveness of the interventions even though these data have not been proven. This lack of data results in all of the tests being dominated in Base case 1. Pragmatic studies assessing the benefits of the interventions in changing real world decisions are required to provide appropriate data.

Conclusions

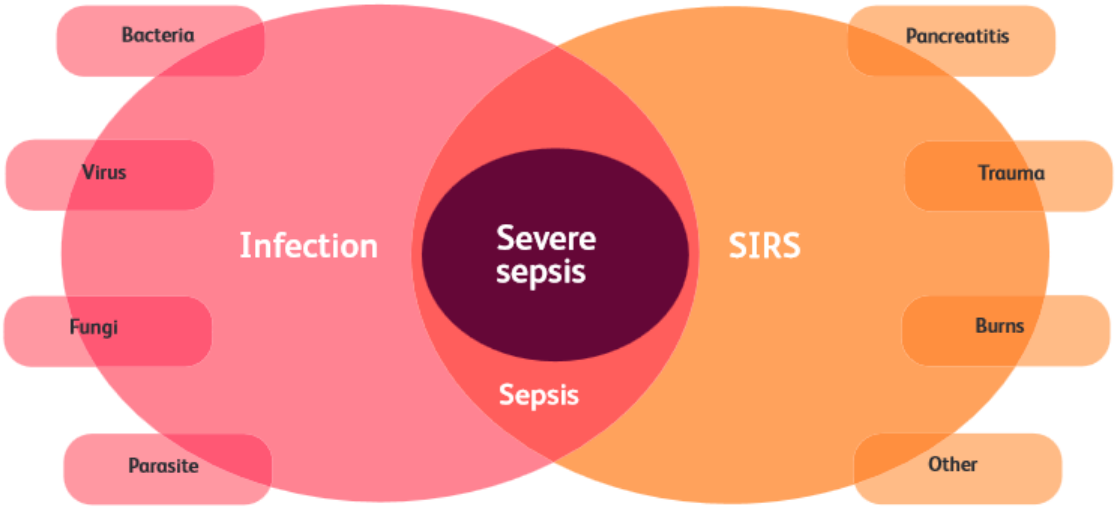
Based on the current evidence, no definitive conclusions regarding either the clinical or cost effectiveness of the interventions can be made. However, evidence based on expert clinical judgement suggests that the tests are likely to be beneficial to patients but this needs to be proven within appropriate studies.

1. BACKGROUND AND DEFINITION OF THE DECISION PROBLEM

1.1 Background to sepsis and blood stream infection

Sepsis is a condition characterised by the body's inflammatory response to an infection. Sepsis is diagnosed where there is evidence of systemic inflammation, in addition to a documented or presumed blood stream infection. Systemic illness often occurs when bacteria or fungi invade normally sterile parts of the body. One example of this is the invasion of bacteria or fungi into the blood stream, a process which often causes an inflammatory immune response. A pictorial representation of the relationship between systemic inflammatory response syndrome (SIRS), infection, sepsis and severe sepsis is provided in Figure 1. This is a reproduction of a diagram produced by the Royal College of Physicians.[1]

Figure 1: The relationship between systemic inflammatory response syndrome (SIRS), infection, sepsis and severe sepsis[1]



If sepsis is not treated with antibiotics it can progress to severe sepsis or septic shock and can lead to multiple organ failure and death. Severe sepsis occurs when the body's response to infection interferes with the functioning of vital organs, such as the heart, kidneys, lungs or liver. Severe sepsis has historically been defined as infection and at least two SIRS criteria,[2] however a recent paper suggests that the need for two or more SIRS criteria excluded one in eight patients with infection, organ failure and substantial mortality risk.[3] SIRS criteria are: fever of more than 38°C or less than 36°C; a heart rate of more than 90 beats per minute; respiratory rate of more than 20 breaths per minute or arterial carbon dioxide tension of less than 32mm Hg; abnormal white blood cell count ((>12,000/µL or < 4,000/µL or >10% immature [band] forms).[4]

Septic shock occurs in severe cases of sepsis, and is defined as persistent sepsis-induced hypotension (low blood pressure) despite adequate fluid resuscitation. Septic shock prevents organs from receiving enough oxygenated blood. Complications of septic shock can include: respiratory failure; heart failure; kidney injury or failure; and abnormal blood clotting. Severe sepsis is a time-critical condition where delays in recognition and the subsequent administration of appropriate treatment can adversely impact on outcomes. It has been reported that the survival rate of untreated patients with sepsis decreases by the hour.[5]

The cost implications of sepsis are considerable. The consequence in terms of mortality and morbidity is large with Levy *et al.*,[6] reporting a mortality rate of 46% for septic patients with both hypotension and lactate $\geq$ 4 mmol/L. However, compliance with the 2004 Surviving Sepsis Guidelines[7] appears to reduce both mortality and length of stay outcomes: Levy *et al.*,[8] report that mortality was lower (29.0%) in those with high compliance with the resuscitation bundle compared with those with low compliance (38.6%). Hospital mortality rates dropped 0.7% for every 3 months of participation with the campaign and hospital and intensive care length of stay decreased 4% for every 10% increase in site compliance: all of these reductions were statistically significant. An estimate of mortality in patients with early septic shock was 29% at 90-days.[9] Lower estimates of mortality have been provided in a recent study of patients with hospital-acquired infection with 13% mortality at 28 days,[10] and in data from Australia and New Zealand which report in-hospital mortality as approximately 10% for SIRS-positive sepsis and 20% for SIRS-negative sepsis.[3]

Severe sepsis is one of the most common reasons for admission to a critical care unit, accounting for almost one third of all admissions. In the UK sepsis is estimated to be responsible for 100,000 hospital admissions and 37,000 deaths per year.[11]

Bacterial infections are the most common cause of blood stream infection; however they can also be caused by viral and fungal infections. The most common sites of infection leading to sepsis are the lungs, urinary tract, abdomen and pelvis. Other sources of infection leading to sepsis include skin infections (such as cellulitis), post-surgical infections and infections of the nervous system (such as meningitis or encephalitis). Bacteria can be categorised into three groups: Gram-positive bacteria, Gram-negative bacteria and very rarely Gram-indeterminate bacteria.

Patients who are currently or have recently been hospitalised, are at risk of acquiring a healthcare associated infection and are therefore at increased risk of sepsis and bloodstream infection. It is thought that the increasing number of invasive procedures (such as catheterisation), immunosuppressive therapy, antibiotic therapy and life support measures has resulted in an increase in healthcare associated blood stream infections (Public Health England 2014a[12]). In 2011, an estimated 6.4% (95% Confidence Interval (CI) 4.7 – 8.7%) of patients in acute care hospitals were

diagnosed with a healthcare associated infection, with the largest proportion, 23.4% within the ICU.[13] Of patients with a healthcare associated infection it was estimated that 7.6% had a blood stream infection.[13] Septic shock is most commonly associated with Gram-negative bacterial blood stream infections, but shock can also be associated with blood stream infections caused by Gram-positive bacteria, particularly with fulminant pneumococcal, Lancefield Group A streptococcal and Staphylococcus aureus infections (Public Health England 2014b[14]). Community acquired blood stream infections occur in people who have not had recent contact with healthcare services. The spectrum of pathogens isolated from these people may differ from those associated with healthcare acquired blood stream infection (Public Health England 2014a[12]).

Blood stream infection is also a risk for people who are immunocompromised, particularly amongst people with neutropenia, who are at risk of developing neutropenic sepsis. People who are immunocompromised have a higher incidence of infections caused by pathogens that pose low risk to those whose immune system is not impaired, such as Pseudomonas species, *Listeria monocytogenes*, *Corynebacterium* species, *Candida* species, coagulase-negative *staphylococci*, *enterococci* and *viridans streptococci*. Polymicrobial infections are also more common amongst people who are immunocompromised (Public Health England 2014a[12]).

The bacteria most commonly associated with bloodstream infection in adults in England, Wales and Northern Ireland are outlined below in Table 1.

Table 1: The bacteria most commonly associated with bloodstream infection in adults in England, Wales and Northern Ireland between April 2011 and March 2012. Adapted from Davies[15]

	Percentage of all bacteria associated with blood stream infection	Group of bacteria
Eschericha coli	36%	-
Staphylococcus aureus (MSSA)	9.7%	+
Klebsiella spp.	7.8%	-
Non-pyogenic streptococci	7.1%	+
Other gram-negative	6.4%	-
Enterococcus spp.	6.3%	+
Pseudomonas spp.	4.3%	-
Streptococcus pneumoniae	4.2%	+
Other gram-positive	4.2%	+
Proteus spp.	3.1%	-
Enterobacter spp.	2.2%	-
Staphylococcus aureus (MRSA)	1.6%	+
Bacteroides spp.	1.5%	-
Group B Streptococci	1.4%	+
Group A Streptococci	1.4%	+
Diphtheroids	1.2%	+
Serratia spp.	1.0%	-
Acinetobacter spp.	0.7%	-

MSSA: methicillin-sensitive *staphylococcus aureus;*
MRSA: methicillin resistant *staphylococcus aureus*
+: Gram-positive; -: Gram-negative

The types of pathogens causing bloodstream infection can also differ slightly in children compared with those isolated from adults with bloodstream infection. Pathogens particularly associated with community acquired blood stream infection in children include *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Staphylococcus aureus*, and *Escherichia coli*. The profile of pathogens associated with healthcare associated infections in children is thought to be similar to that associated with healthcare associated infections in adults; however, polymicrobial infection and anaerobic bacteraemia are thought to occur less frequently amongst children (Public Health England 2014a[12]).

1.2 Diagnosis of sepsis

Diagnostic criteria for sepsis are listed in the Surviving Sepsis Campaign guidelines[16] (adapted from Levy *et al.*, 2003[17]). In summary, regular observations of all vital signs should be taken and recorded, kidney and liver function tests should be performed, inflammatory biomarkers and serum lactate should be measured. These guidelines state that a diagnosis of sepsis should be based on infection, documented or suspected, in conjunction with hyperthermia or hypothermia, tachycardia and at least one indication of altered organ function (see bullet point below). The diagnostic criteria for sepsis include the following variables:

- General variables: temperature of greater than 38.3°C or less than 36°C; heart rate greater than 90 beats per minute; rapid breathing, altered mental status; significant oedema; high blood sugar in the absence of diabetes.
- Inflammatory variables: low or high white blood cell count or more than 10% immature forms; raised plasma CRP; raised plasma procalcitonin.
- Haemodynamic and tissue perfusion variables: low blood pressure; raised blood lactate (a concentration of ≥4mmol/l suggests tissue hypoperfusion).
- Organ dysfunction variables: low blood oxygen; reduced urine output; increased creatinine levels (indicating impaired kidney function); coagulation abnormalities; absent bowel sounds; reduced platelet count; raised plasma bilirubin levels.

1.3 Current standard of care for patients with suspected blood stream infections or suspected sepsis

The diagnostic work-up of sepsis and blood stream infection is described in several guidelines:

- The National Institute for Health and Care Excellence (NICE) Clinical Guideline 151: prevention and management of neutropenic sepsis in cancer patients (2012[18])
- The Royal College of Obstetricians and Gynaecologists: Green-Top Guideline 64a Bacterial Sepsis in Pregnancy (2012[19])
- The Royal College of Obstetricians and Gynaecologists: Green Top Guideline 64b Bacterial Sepsis following Pregnancy (2012[20])
- Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock (2012[16]).

In addition a NICE clinical guideline 'Sepsis: the recognition, diagnosis and management of severe sepsis' is currently in development with an estimated publication date of July 2016.[21] Furthermore, the Commissioning for Quality and Innovation payment framework (CQUIN) which is currently in development have announced new sepsis mandates to monitor adherence to the sepsis care pathway across the National Health Service (NHS).[22]

The Surviving Sepsis Campaign guidelines make the following specific recommendations relating to the detection of localised and blood stream infection:

- At least 2 sets of blood cultures should be collected (aerobic and anaerobic) before antimicrobial therapy is initiated if such cultures do not cause significant delay (>45 minutes) in the start of antimicrobial administration. At least one should be drawn percutaneously and one drawn through each vascular access device, unless the device was recently (<48 hours) inserted. The blood cultures can be drawn at the same time if they are obtained from different sites. Cultures of other sites such as urine, cerebrospinal fluid, wounds, respiratory secretions

or other bodily fluids that may be the source of infection should be obtained before initiation of antimicrobial therapy, if doing so does not cause significant delay in the start of antimicrobial administration.

- Imaging studies such as CT or X-ray should be performed in order to confirm a potential source of infection.
- Assays to diagnose systemic fungal infection should be used if available and invasive candidiasis is suspected.

The Surviving Sepsis Campaign guidelines recommend care 'bundles' which should be initiated during the diagnostic work-up of a patient. The 3-hour bundle should be completed within 3 hours of a patient developing symptoms which are indicative of sepsis:

a. Measure lactate levels to identify tissue hypoperfusion
b. Obtain blood cultures prior to administration of antibiotics
c. Administer broad spectrum antibiotics
d. Administer 30ml/kg crystalloid for hypotension or lactate ≥4mmol/L

The 6-hour bundle should be completed within 6 hours at presentation in the emergency department or recording of symptoms if in hospital when sepsis starts:

e. Apply vasopressors (for hypotension that does not respond to initial fluid resuscitation) to maintain a mean arterial pressure ≥65mm Hg
f. In the event of persistent arterial hypotension despite volume resuscitation (septic shock) or initial lactate ≥4mmol/L:
- Measure central venous pressure
- Measure central venous oxygen saturation
g. Re-measure lactate if initial lactate was elevated

The treatment of sepsis varies based on the initial infection, the organs affected and the extent of tissue damage. The management of severe sepsis and septic shock is described by the Surviving Sepsis Campaign in their International Guidelines for the Management of Severe Sepsis and Septic Shock (2012).[16] All patients with severe sepsis or septic shock will require initial resuscitation, antimicrobial therapy, source control (where appropriate) and fluid therapy. Some patients may require additional treatment with vasopressors, inotropic therapy, corticosteroids and other supportive therapy.

It is recommended that intravenous empiric antimicrobials should be administered within the first hour of recognition of septic shock and severe sepsis. The initial antimicrobial therapy should include one or more drugs that have activity against all likely pathogens (bacterial and/or fungal or viral) and

that penetrate in adequate concentrations into the tissues presumed to be the source of sepsis (Surviving Sepsis Campaign 2012[16]). Such treatment is typically referred to as being 'broad spectrum'. Frequently used broad spectrum antibiotics for more serious infections include beta-lactams and aminoglycosides. Carbapenems are often the last option in patients with hard to treat infections (Department of Health 2013[23]).

The choice of empirical antimicrobial therapy is often based on:

- the patient's history including drug intolerances
- recent treatment with antibiotics
- underlying disease
- the clinical syndrome
- susceptibility patterns of pathogens in the local community and hospital
- microbiology reports identifying pathogens which have previously colonised or infected the patient

Clinicians should also consider whether a fungus is a likely causative pathogen when selecting initial therapy and administer empirical antifungal therapy when appropriate.

The use of antimicrobials varies between hospitals as prescribing choices are influenced by local resistance and susceptibility patterns. The choice of antimicrobials is also influenced by the suspected source of the infection and local prescribing protocols may be developed for:

- urinary tract infections
- upper respiratory tract infections
- lower respiratory tract infections
- soft tissue infections
- central nervous system infections
- gastrointestinal infections, genital tract infections
- bloodstream infections
- eye, ear, nose and throat infections
- sepsis of unknown origin

1.4 Current practice for detecting pathogens in those with suspected blood stream infection or sepsis

The current practice for detecting pathogens in those with suspected blood stream infection or sepsis consists of clinical assessment in conjunction with blood culture. However, within the NICE scope for this project[24] an additional comparator of clinical assessment in conjunction with blood culture and matrix-absorbed laser desorption/ionization- time of flight (MALDI-TOF) mass spectrometry (MS)

was included in recognition of the fact that some hospitals are incorporating MALDI-TOF MS within their standard practice. MALDI-TOF MS has an advantage of shortening the time required for identifying the causative pathogen when a blood culture becomes positive.

1.4.1 Blood culture

Blood culture is required for the detection and subsequent identification of bloodstream bacteria and fungi, and to provide potential definitive antimicrobial susceptibility data. Standards for the investigation of blood cultures are available from Public Health England (2014a[12]). A blood culture set for the diagnosis of blood stream infection is defined as one aerobic and one anaerobic bottle (Public Health England 2014a[12]). For adult patients it is recommended that 20-30ml of blood be cultured per set, and that two consecutive blood culture sets from two separate sites should be collected during any 24 hour period for each septic episode. The first set should be taken prior to the administration of antimicrobial treatment as the presence of antibiotics or antifungals may inhibit the growth of pathogens in the blood culture (Public Health England 2014a[12]). Blood culture bottles should be incubated within 4 hours of the blood sample being taken with many laboratories now using automated culture systems such as the BACTEC or Bact/ALERT systems, which alert laboratory staff once growth has been detected.

The time taken for a blood culture bottle to show positivity is variable and can depend on the individual pathogen, the volume of cultured blood, the concentration of organisms in the sample, whether there are multiple pathogens, and whether the patient had recently received antibiotics prior to the blood being sampled.[25,26] A median time to positivity of approximately 15 hours has been reported but with a wide range for individual samples.[25,26]

When a blood culture bottle has been detected as positive it is recommended (Public Health England 2014a[12]) that:

- Gram staining and rapid antigen testing should be performed within 2 hours.
- Direct or automated isolate identification should be performed within 24 hours (extending to 48 hours if traditional microbiology techniques such as morphological identification are used). Rapid species identification may be done following blood culture using techniques such as MALDI-TOF MS.
- Identification should be followed by susceptibility testing to determine to which antimicrobials the identified pathogen is susceptible. A preliminary report should be made within 24 hours.
- A preliminary positive report is made within 2 hours of identification and susceptibility testing, and a final positive report should be made within 5 days of the sample arriving in the laboratory.

These first target is not typically met by laboratories as if the blood culture is detected as positive during the night gram staining would not occur until the laboratory opened in the morning.

If a blood culture is not positive within 48 hours of sample receipt in the laboratory it is recommended that a preliminary negative report is provided with a final negative report issued within 5 days unless extended culture is being undertaken for example if fungi or unusual, fastidious or slow growing organisms are suspected (Public Health England 2014a[12]).

Blood culture results may not detect pathogens within an individual's bloodstream due to the transient nature of blood stream infections and a low number of organisms present in a blood sample; there can often be fewer than $1x10^3$ colony forming units per litre in adults with blood stream infection (Public Health England 2014a[12]). The presence of antibiotic treatment prior to the blood being sampled can also result in pathogens not being detected. Conversely, blood culture results may identify a pathogen that is not within an individual's bloodstream when pathogens transferred from the skin during the drawing of blood contaminate the culture. To reduce the incidence of such false positive results current standards recommended that contamination rates are no higher than 3% (Public Health England 2014a[12]). In addition, several criteria may be used to differentiate between contamination and true blood stream infection which include: the identity and clinical significance of the pathogen; the number of positive blood culture sets and positive culture bottles; and the quantity of growth detected.

Blood culture sample collection differs for infants and neonates, for whom a single aerobic bottle or low volume blood culture bottle maybe requested (Public Health England 2014a[12]). Criteria for calculating total blood culture volumes in neonates and children are based on weight rather than age and relate to total patient blood volume. It has been suggested that the volume of blood drawn should be no more than 1% of the patient's total blood volume (Public Health England 2014a[12]). In infants and children the magnitude of bacteraemia is usually higher than that in adults and therefore the sensitivity of detection is not believed to be significantly reduced by lower blood-to-medium ratio (Public Health England 2014a[12]).

Whilst blood culture is considered the gold standard a number of limitations regarding its use were identified, for example it has been estimated that only 30-60% of blood cultures taken from patients with sepsis are positive.[27] This may indicate poor sensitivity which may be attributed to commencement of antimicrobial therapy prior to sample collection, low pathogen levels in blood, and inadequate blood sampling. Additionally, blood culture does not always pick up fungal pathogens.[28]

1.4.2 MALDI-TOF MS

Following a blood culture becoming positive it is possible to use MALDI-TOF MS to provide an identification of the pathogen more quickly than by standard phenotypic techniques alone. Details on

MALDI-TOF MS have been provided by Schubert *et al.*,[29] where pathogens were identified from an agar plate. Recently however, Sepsityper, a preparation method prior to MALDI-TOF MS, has been developed allowing MALDI-TOF MS to be used directly on a positive blood culture bottles without the need for growing pathogens on an agar plate. The use of Sepsityper can thus provide a result more quickly than standard culture-based identification techniques or MALDI-TOF MS used in conjunction with agar plates. Using Sepsityper, Morgenthaler and Kostrzewa[30] report that 'the use of the Sepsityper sample preparation kit leads to a reduction in overall time to results from 8 to >48 hours (in some studies >100 hours), depending on the microorganism growth rate on solid phase culture plates'. The level of Sepsityper use in England is currently unknown.

A recently completed National Institute for Health Research funded study RAPIDO (A prospective randomised, multicentre trial to assess the impact of laboratory based rapid diagnosis on outcome in patients with blood stream infections) has compared MALDI-TOF MS to standard practice having recruited 4536 patients from the UK.[31] However, at the time of writing the data analysis had not been fully conducted. The primary outcome measure within the RCT is the 28-day all-cause mortality between the two arms. Following personal communication with Dr Leeming (Clinical Scientist, North Bristol NHS Trust), 15 June 2015, it was identified that Sepsityper had been used in the MALDI-TOF MS arm in all centres bar Newcastle, where the centre used its own method.

1.5 The risk of antimicrobial resistance

Broad spectrum antibiotics administered to patients with suspected sepsis are a mainstay of treatment; however, these interventions cannot be used indiscriminately without risking unwanted consequences. Antimicrobial resistance describes the development of resistance to existing antimicrobial medications (including antibiotics, anti-fungals and anti-virals) amongst bacteria, viruses and fungi. As existing antimicrobial medications are becoming less effective, strategies such as the UK five year antimicrobial resistance strategy (Department of Health 2013[23]) have been introduced to help conserve the effectiveness of existing treatments. One of the key priorities outlined in the UK five-year antimicrobial resistance strategy is the introduction of antimicrobial stewardship programmes which aim to promote the rational prescribing of antimicrobial medications and the use of existing and new rapid diagnostic tests.

Recent surveillance data for England suggest that rates of methicillin-resistant Staphylococcus aureus have fallen whilst there is an increase in the incidence of bloodstream infections caused by resistant Enterobacteriaceae (Gram-negative bacteria) such as *Klebsiella species* and *Escherichia coli*. Of particular concern in some regions of England, such as the North West and Greater London, is the increasing resistance to carbapenem antibiotics which are often used as a last resort for treating severe infections.

Clinicians prescribing antimicrobial therapy should take into account the Department of Health's guidance on antimicrobial stewardship which is based on the "start smart then focus" strategy (Department of Health 2011[32]). The guidance recommends that, when antimicrobials are administered empirically, the patient is reviewed after 48 to 72 hours to allow an "antimicrobial prescribing decision" to be made. This decision should take into account available microbiology results to determine whether therapy can be stopped or changed, that is, the de-escalation, substitution or addition of antimicrobial agents to the treatment plan (Department of Health 2011[32]). Narrowing the spectrum of antimicrobial coverage and reducing the duration of therapy is thought to be associated with a reduction in the risk of a patient developing a superinfection, a reduction in the selection of resistant organisms and a reduction in treatment related side-effects. Adverse events associated with the use of broad spectrum antimicrobials may include diarrhoea, nausea, vomiting, hearing loss, damage to the kidneys and an increased risk of developing superinfection with *Clostridium difficile*.

Narrowing the spectrum of antimicrobial coverage may also be associated with an increase in treatment efficacy as certain broad spectrum antibiotics may not be as effective as related narrow spectrum antibiotics against certain pathogens (Department of Health 2011[32]). In addition, a reduction in agents may result in costs savings.

The National Institute for Health and Care Excellence (NICE) recently issued a draft clinical guideline on antimicrobial stewardship which discussed the evidence for de-escalation of antimicrobials.[33] A conclusion of this draft guideline was that five randomised controlled trials (RCTs) had assessed the impacts of de-escalation, although only three are explicitly referenced,[34-36] four of which were set in intensive care units (ICU), the exception being hospital-based, and only one of which, Leone *et al.*,[35] was in patients with sepsis. The Guideline Development Group (GDG) found no evidence from these RCTs that de-escalation between 48 and 72 hours increased patient mortality. The GDG found little evidence of increased length of ICU or hospital stay but noted the exception of Leone *et al.*,[35] which was classified as a low quality RCT, which recruited 116 with severe sepsis who were randomised to de-escalation or continuation of empirical antimicrobial treatment. Leone *et al.*,[35] reported statistically significantly greater rates of superinfection in the de-escalation group (27% vs 11%; p-value = 0.03) and in the mean number of antibiotic days (9 vs 7.5; p-value = 0.03) although the increase in median duration of ICU stay (9 days vs 8 days) was not statistically significant (p-value = 0.71). The GDG noted that it identified no health economic evidence regarding which interventions, systems and processes are effective or cost effective in reducing antimicrobial resistance without causing harm to patients, nor did it identify any health economic evaluations that included outcomes of antimicrobial resistance.

We have used the term ICU throughout the report as this is the term often used within the published literature although we recognise that care can also be provided in other critical care settings. We have assumed that such settings are encompassed within the ICU categorisation.

The External Assessment Group note that clinical advice received during the scoping process stated that a barrier to de-escalation in practice could be the resistance of family members to change the treatment in a patient who was clearly improving and thus it is unclear the extent to which de-escalation would occur in clinical practice.

1.6 The potential benefits and possible harms of a test that could provide earlier information on pathogen

The individual characteristics of the three tests evaluated in this report (LightCycler SeptiFast Test MGRADE®, SepsiTest™ and IRIDICA BAC BSI assay) are detailed in the following section. The aim of this section is to explain the benefits that could be provided by tests that report information on the type of bacteria earlier than standard blood culture methods, with or without MALDI-TOF MS which can be used with or without Sepsityper. Were a rapid test to have a sensitivity of 100% and a specificity of 100% in identifying the pathogen(s), caused by blood stream infection, that is, the test was perfect, management strategies could be quickly altered dependent on whether there was presence of a pathogen. Were a pathogen to be identified then treatment could be tailored to that pathogen alongside de-escalation of antimicrobial treatment by removing the components of broad spectrum treatment to which either the pathogen was not sensitive, or to which a targeted treatment was more effective. Were a pathogen not identified then treatment could be de-escalated, or removed entirely. Due to the rapid identification by the test these benefits would be achieved more quickly than through standard techniques.

The advantages of earlier appropriate treatment have been reported in the published literature. A Spanish retrospective matched cohort study[37] attempted to determine the attributable mortality and excess length of stay associated with inadequate empirical antimicrobial therapy between 1997 – 2006. Therapy was considered inadequate when no effective drug against the isolated pathogen(s) was included in the empirical antibiotic treatment within the first 24 hours of admission to the ICU, or the doses and pattern of administration were not in accordance with current medical standards. From 87 matched pairs 59 (67.8%) died in the inadequate group compared with 25 (28.7%) in the control group. Removing pairs with nosocomial infection still showed a 31.4% excess in mortality (65.7% vs 34.3%). In those without a nosocomial infection there was a significant reduction in the length of stay in ICU associated with adequate treatment (7 vs 9 days; p-value = 0.02).

Using a generalised linear model, adjusted for confounders, Zilberberg *et al.*,[38] estimated that the excess length of hospitalisation was 7.7 days (95% CI 0.6-13.5%) and excess costs were $13,398

(95% CI $1,060-$26,736) when a patient had inadequate antifungal treatment. Inadequate antifungal treatment was defined as treatment delay of ≥24 hours from Candidemia onset or inadequate dose of an antifungal agent active against the pathogen.

Arnold *et al.*,[39] attempted to estimate from 167 consecutive patients the costs of inappropriate treatment of Candidemia, which was defined as delayed antifungal therapy >24 hours from culture collection. 22 patients had appropriate therapy, 145 did not. Length of stay was shorter in the appropriately treated group (7 vs 10.4 days; p-value = 0.037) and the costs were lower ($15,832 vs $33,021; p-value <0.001).

Morrell *et al.*,[40] retrospectively analysed 157 consecutive patients over a 4-year period with a candida bloodstream infection of which 50 (32%) died during hospitalisation. The number of people without a delay in antifungal treatment (>12 hours) was 9, whilst 148 patients had delayed treatment. The adjusted odds ratio associated with delay in antifungal treatment was 2.09 (95% CI 1.53-2.84). Delays in antifungal treatment were also associated with a longer duration within ICU (9.4 days vs 0.4 days; p-value = 0.019).

It is unlikely that the tests evaluated would be 100% sensitive and 100% specific, meaning that the consequences of misdiagnoses would also need to be considered. These take the form of false positives (where a pathogen is identified that is not present) and false negatives (where a pathogen is not identified that is present in the blood culture). The consequences of these misdiagnoses are likely to differ. For false positives there is the risk of over-treatment which would incur cost and could increase the risk of antimicrobial resistance; for false negatives, if treatment was withdrawn the patient could be at increased risk of morbidity and mortality.

However, it is known that diagnostic inaccuracy is not confined to the new tests and can occur in standard techniques and that, as such, standard techniques provide an inaccurate gold standard which may result in biased evaluation of the interventions. This is believed most likely where the correct identification of a pathogen could be classed as a false positive if it was not detected by blood culture. As detailed within this report some clinical experts believe that such results would provide valuable information in the patient decision treatment despite adversely affecting the specificity of the test against blood culture.

1.7 Description of the technologies under assessment

Our research aims to evaluate the clinical and cost effectiveness of three tests which potentially allow the rapid detection and identification of bacterial and fungal deoxyribonucleic acid (DNA) present in the blood stream of people who are suspected of having sepsis. These tests are: the LightCycler SeptiFast Test MGRADE®; SepsiTest™; and IRIDICA BAC BSI assay which will be compared with

blood culture, with or without, MALDI-TOF MS. Each test is intended to be run directly on whole blood samples without prior incubation or pre-culture steps, allowing an earlier initial assessment of the patient. It is anticipated that blood cultures and clinical judgement would be required conjunction with each test to provide additional, potentially more definitive data, on the most effective antimicrobial to use as data on this provided by the interventions are very limited. This section details the three technologies with the comparators having been described in Section 1.4. For brevity where the test name alone is provided it should be assumed that this denotes being used in conjunction with blood cultures and clinical judgement. Similarly any reference to blood culture, with or without MALDI-TOF MS also denotes these being used in conjunction with blood cultures and clinical judgement.

1.7.1 LightCycler SeptiFast Test MGRADE®

LightCycler SeptiFast Test MGRADE® (Roche Diagnostics) – henceforth referred to as SeptiFast - is a CE-marked in-vitro diagnostic real-time polymerase chain reaction (PCR) test which simultaneously detects and identifies bacterial and fungal DNA. The test requires 1.5ml of ethylenediaminetetraacetic acid (EDTA)-treated whole blood which can be processed without prior incubation or culturing. SeptiFast involves three distinct processes: specimen preparation by mechanical lysis and purification of DNA; real-time PCR amplification of target DNA in 3 parallel reactions (gram-positive bacteria, gram-negative bacteria, fungi); and detection using fluorescence labelled probes specific to the target DNA. The test takes around 6 hours in optimal conditions, but could take longer depending on laboratory workflow.

The SeptiFast Identification Software set v2.0 analyses the samples and generates a report including relevant laboratory data and details of the identified species. The software also includes a crossing point cut-off rule which is intended to reduce the positive rate for Coagulase negative Staphylococci and *Streptococcous* spp. based on the assumption that they are contaminants and not causal agents when the crossing point value is less than 20.

Where *Staphylococcus aureus* is identified in a sample, an aliquot of the SeptiFast Test MGRADE eluate can be further tested for the presence of the MecA gene using the LightCycler SeptiFast MecA Test MGRADE. The test is intended to determine the likely methicillin resistance of the *Staphylococcus aureus* through PCR using the LightCycler 2.0 instrument.

The bacteria and fungi species which can be detected by SeptiFast are shown in Table 2.

Table 2: Bacteria and fungi species detected by the LightCycler SeptiFast Test MGRADE

Bacteria		Fungi
Gram-negative	**Gram-positive**	
Escherichia coli	Staphylococcus aureus	*Candida albicans*
Klebsiella (pneumonia/oxytoca)	Coagulase negative *Staphylococci* (including *S. epidermidis*, *S. haemolyticus*)	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter (cloacae/aerogenes)	*Streptococcus* spp. (including *S. pyogenes, S. agalactiae, S. mitis*)	*Candida krusei*
Proteus mirabillis	*Enterococcus faecium*	*Candida glabrata*
Pseudomonas aeruginosa	*Enterococcus faecalis*	Aspergillus fumigatus
Acinetobacter baumannii		
Stenotrophomonas maltophilia		

Species often referred to as A. calcoaceticus-A. baumannii (Acb complex) are not detected

The test has an analytical sensitivity of 100 colony forming units/millilitre for coagulase negative *Staphylococci*, *Streptococcus agalactiae*, *Streptococcus pyogenes, Streptococcus pneumonia* and *Streptococcus mitis*. The minimum analytical sensitivity for all other pathogens detected by SeptiFast is 30 colony forming units/millilitre.

1.7.2 SepsiTest™

SepsiTest™ (Molzym Molecular Diagnostics) – henceforth referred to as SepsiTest - is a CE-marked PCR test for detecting bacterial and fungal DNA in 1ml k-EDTA-or citrate-treated whole blood. The test is able to identify species from more than 200 genera of bacteria and 65 genera of fungi. The manufacturer states that SepsiTest can identify *Candida krusei* although this organism has not been found in any study to date.

SepsiTest involves 3 distinct processes: extracting and purifying microbial DNA using centrifugation; universal PCR; and Sanger sequencing. The PCR result is available after 4 hours in optimal conditions although this could take longer dependent on laboratory workflow, indicates whether bacteria or fungi are present in the sample. Amplicons from positive samples are then sequenced to confirm the PCR result and to determine which bacteria or fungi species are present. Where readable sequences are available from sequence analysis, bacteria and fungi can be identified using the SepsiTest-BLAST online tool. Sequencing results is typically available in 3-4 hours in optimal conditions, depending on the analyser used, equating to at time of 8 hours from drawing blood, but could take longer based on laboratory workflow.

The analytical sensitivity of SepsiTest ranges from 10 to 80 colony forming units per millilitre, depending on the target species.

Shortly before the submission of this report Molzym Molecular Diagnostics informed NICE on the 22nd of July 2015 that they had updated SepsiTest to version 4.0 [date of change 1st of July 2015]. The changes reported by the company include: the implementation of an internal extraction control to validate the extraction of the DNA; the removal of the internal control from the kits; and that it was no longer recommended to process duplicate samples. In consultation with NICE a decision was taken to exclude the updated version of SepsiTest from the analyses conducted in this report primarily because there were no data provided on the diagnostic accuracy associated with this version. Given the potentially large change compared with the previous version regarding the removal of the duplicate sample it could not be assumed without supportive evidence that the results from previous studies were applicable to the latest version of SepsiTest.

1.7.3 IRIDICA BAC BSI

The IRIDICA BAC BSI assay (Abbott Diagnostics) – henceforth referred to as IRIDICA - is a CE-marked in-vitro diagnostic test for detecting and identifying bacteria and candida DNA in 5ml EDTA-treated whole blood. The test can also detect the mecA (Staphylococcus specific methicillin resistance), vanA and vanB (Enterococcus specific vancomycin resistance) and KPC (gram-negative associated carbapenem resistance) genes which are associated with antibiotic resistance. The test is designed for use with the IRIDICA system which combines broad range PCR with electrospray ionisation time of flight mass spectrometry to amplify and detect pathogens. The IRIDICA system includes a proprietary database and software which identifies the organism present in the sample by comparing the sequence of the sample with a library of known sequences. The IRIDICA system was developed incrementally from a previous test called PLEX-ID (Abbott Diagnostics) although the final IRIDICA system has key differences from PLEX-ID as it uses a greater volume of whole blood (5mL compared with 1.5mL) and has different desalter and mass spectrometry modules. The company supplied commercial-in-confidence data regarding the equivalency of IRIDICA and PLEX-ID which the company declared demonstrated that the limits of detection of 4 core organisms were comparable between IRIDICA and PLEX-ID. Based on these data the External Assessment Group were comfortable with including data from studies that used IRIDICA-PLEX-ID hybrid systems.

The IRIDICA assay is able to detect over 780 bacterial and candida species. The mean limit of detection for the assay is 39 colony forming units per millilitre, with a range of 0.25 to 128 colony forming units per millilitre depending on the target species. The estimated time to result is 5 hours and 55 minutes in optimal conditions, although this may take longer based on laboratory workflow.

1.8 The Decision Problem

This report aims to evaluate the clinical, and cost, effectiveness of the three interventions in comparison with blood culture, with or without MALDI-TOF MS. As detailed in Section 1.6 there are reasons to believe that a quicker identification of pathogens can produce health benefits. The quickest

time at which clinically important information would be available for each test is provided in Section 1.8.1.

1.8.1 An estimation of the time to clinically important information associated with each intervention and comparator

Table 3 denotes estimations of time to clinically relevant events in the detection of pathogens associated with bloodstream infections. It is noted that for the interventions it has been assumed that workflow is optimal, i.e. that the test result will be reported back in a timely manner and not delayed either due to staff hours, waiting for additional blood to be gathered which will be tested simultaneously, or transport times. For the comparators the time of day has been included within the estimates to produce a range of possible time to event data. As such, the timings presented in Table 3 are favourable to the interventions.

Table 3: Estimated time to clinically relevant events associated with the interventions and the comparators

Test	Time to Indication of whether bacteria of fungi are present (hours) for SepsiTest † or Time to indication of gram stain positive or gram stain negative in positive cultures	Time to preliminary identification of type of organism	Time to preliminary antimicrobial sensitivity data	Time to earliest possible identification of precise bacteria or fungi † Δ
Interventions				
SeptiFast				6
IRIDICA BAC BSI				6
SepsiTest	4 hours [henceforth denoted A]			[A] + 3 to 4 hours. Range: 7 - 8 hours
Comparators				
Blood culture	15 hours (range 12 to 48 hours)* [henceforth denoted B]	[B] + 12 to 24 hours** [henceforth denoted C]	[C]	[C] + 12 to 18 hours *** Range: 36 to 90
Blood culture with MALDI-TOF MS	[B]	[C]	[C]	[C] Range: 24 to 72 hours
Blood culture with MALDI-TOF MS and Sepsityper	[B]	[B] + 1 to 13 hours**	[C]	[C] Range: 24 to 72 hours

† Assuming optimal workflow conditions for the interventions. These times may be elongated given work patterns and location of the required equipment

Δ Note that a subsequent identification based on blood culture methods will also become available

* Based on the time at which a blood culture bottle flags positive

** Positive blood cultures have been sub-cultured on agar plates. The time taken is dependent on the time of day of blood culture positivity

*** The time taken is based on the speed of bacterial growth

2. ASSESSMENT OF CLINICAL EFFECTIVENESS

A systematic review of the literature and meta-analysis (where appropriate) was undertaken to evaluate the clinical-effectiveness of the SeptiFast, SepsiTest and IRIDICA assays in conjunction with clinical assessment for rapidly identifying bloodstream bacteria and fungi.

A review and meta-analysis was undertaken in accordance with the guidelines published by the Centre for Reviews and Dissemination (CRD) for undertaking systematic reviews[41] and the Cochrane Diagnostic Test Accuracy Working Group on the meta-analysis of diagnostic tests.[42,43]

2.1 Methods for reviewing effectiveness

2.1.1 Identification of studies

a) Electronic databases

Studies were identified by searching the following electronic databases and research registers:

- MEDLINE(R) In-Process & Other Non-Indexed Citations and MEDLINE(R) (OvidSP) 1948 to May 2015
- EMBASE (OvidSP) 1980 to May 2015
- Cochrane Database of Systematic Reviews (Wiley Online) 1996 to May 2015
- Cochrane Central Register of Controlled Trials (Wiley Online) 1898 to May 2015
- Health Technology Assessment Database (Wiley Online) 1995 to May 2015
- Database of Abstracts of Review of Effects (Wiley Online) 1995 to May 2015
- Science Citation Index Expanded (Web of Science) 1899 to May 2015
- Conference Proceedings Index-Science (Web of Science) 1990 to May 2015
- WHO International Clinical Trials Registry Platform (ICTRP) 2007 to May 2015
- Current Controlled Trials (CCT) 2000 to May 2015
- NIH ClinicalTrials.gov 2000 to May 2015
- Manufacturer and User Facility Device (MAUDE) 1991 to May 2015
- MEDION database

Sensitive keyword strategies using free text and, where available, thesaurus terms using Boolean operators and database-specific syntax were developed to search the electronic databases. Synonyms relating to the condition (e.g. sepsis) were combined with terms for the test (e.g. SeptiFast, SepsiTest and IRIDICA). No language restrictions were used on any database; however, the clinical effectiveness searches were date-restricted. To date, all included rapid molecular tests (SeptiFast, SepsiTest and IRIDICA assay) have received a CE mark for use on whole blood samples. For the SeptiFast test, clinical studies on whole blood samples were first published in abstract form by Raglio *et al*., in 2006[44] with subsequent full-text peer-reviewed publications by Mancini *et al*., and Louie *et*

al., in 2008.[45,46] The SeptiFast test gained its CE mark in 2006. For the SepsiTest assay, studies evaluating the use of SepsiTest on whole blood samples in the clinical setting were first published in abstract form by Disqué *et al*., in 2008[47] with a subsequent full-text peer-reviewed publication by Wellinghausen *et al*., in 2009.[48] SepsiTest received a CE mark in 2008. For the IRIDICA assay, studies evaluating the use of IRIDICA on whole blood samples in the clinical setting were first published by Bacconi *et al*., in 2013,[49] which used an IRIDICA-PLEX-ID hybrid system. The final version of the IRIDICA platform received a CE mark in 2014 and has been available for purchase by the NHS since 16th November 2014. Based on these data the clinical effectiveness searches were limited by date from 2006 to May 2015. The search strategy of the current review updated the search strategy of an existing review on SeptiFast[50] and amended it within the scope of the current review (i.e. the search strategy was amended to include generic, trademark or other product names of all the relevant index tests, other bacterial or fungal gene terms were added and were combined with PCR and population terms and a limit to exclude all only animal studies was introduced). An example of the MEDLINE search strategy is provided in Appendix 1.

b) Other resources

To identify additional published, unpublished and ongoing studies, the reference lists of all relevant studies were checked and a citation search of relevant articles (using the Web of Science Citation Index Expanded and Conference Proceedings Citation Index - Science) was undertaken to identify articles that cite the relevant articles. In addition, systematic keyword searches of the World Wide Web (WWW) were undertaken using the Google search engine, key experts in the field were contacted and company submissions were screened for published or unpublished data additional to those identified in studies retrieved from the literature search.

All identified citations from the electronic searches and other resources were imported into and managed using the Reference Manager bibliographic software, (version 12.0; Thomson Reuters, Philadelphia, PA).

2.1.2 Inclusion and exclusion criteria

The inclusion of potentially relevant articles was undertaken using a three-step process. First all titles were examined for inclusion by one reviewer (LU). Any citations that clearly did not meet the inclusion criteria (e.g. non-human, unrelated to sepsis) were excluded. Second, all abstracts were examined independently by two reviewers (LU and AP) and the full manuscript of all potentially eligible articles that were considered relevant was obtained, where possible. Third, two reviewers independently assessed the full-text articles (n=177) for inclusion (LU and AP). All potential included studies (n=87) were then adjudicated by three clinical experts independently (GP, PD and RM). Any disagreements in the selection process were resolved through discussion and included by

consensus between the two reviewers and three clinicians. The relevance of each article for the systematic review was assessed according to the following criteria:

a) Study design

All clinical diagnostic accuracy studies that evaluated the index test with standard culture results (with or without MALDI-TOF MS) on patients' whole blood samples during the management of suspected sepsis were included. In reviews of test accuracy the 'index test' (the test whose performance is being evaluated) can be viewed as the intervention.

Reviews of primary studies were not included in the analysis but were retained for discussion and identification of additional studies. Moreover, the following publication types were excluded from the review: animal models; biological studies; narrative reviews, editorials and opinions; case reports; non-English-language papers and reports published as meeting abstracts only when insufficient methodological details are reported to allow critical appraisal of study quality.

b) Population

All studies of adults and children (of any age) with suspected blood stream infections in secondary care (i.e. departments and wards providing care for acutely unwell patients and/or critical care units) who required blood cultures were included. Potential subgroups of interest included: people with a suspected health care associated infection, people with a suspected community acquired infection, children and neonates, people who are immunocompromised and people exposed to antibiotics prior to blood sample collection. Following clinical advice, people with febrile neutropenia were also considered as potential subgroup of interest. This group of patients usually undergo blood culture testing as their ability to show the classical signs of sepsis are impaired and failing to treat an underlying infection can result in mortality. This practice is supported by a recent large retrospective study by Kaukonen *et al.* (2015)[3] which found that a significant number of poor outcomes from severe systemic infection occur in the absence of SIRS criteria at inception.

c) Target conditions

Suspected sepsis, including severe sepsis and septic shock as defined by Levy *et al*., (2003).[17]

d) Interventions (Index test)

The following tests (in conjunction with clinical assessment) performed on whole blood samples for the detection of bloodstream bacterial and fungal pathogens were included:

- SeptiFast
- SepsiTest

- IRIDICA assay (extended to include preceding versions of the test if the authors believed that the data were likely to be generalisable to IRIDICA assay)

e) Comparator test (Reference standard)

The reference tests included current standard care to define the target condition, which included blood culture (in conjunction with clinical assessment) for the identification of bloodstream bacterial and fungal pathogens with or without MALDI-TOF MS. Where studies were identified that included more than one intervention then these would also form comparators for each intervention.

f) Outcomes

The outcomes of the review included a range of intermediate measures (such as diagnostic accuracy, discordant results with blood culture, time to result, time to treatment, test failure rates, duration of ICU and/or hospital stay, duration of broad and narrow spectrum antimicrobial therapy, re-admission rate and change in antimicrobial treatment plan) and clinical outcome measures (such as side-effects associated with broad spectrum antimicrobial use, morbidity and mortality, severity of disease [as measured by scoring systems such as the Acute Physiology and Chronic Health Evaluation, (APACHE) II; Simplified Acute Physiology Score, (SAPS) II and the Sequential Organ Failure Assessment, (SOFA)], rates of superinfection [including C. difficile], rates of resistant infections and health related quality of life), where available.

2.1.3 Data abstraction strategy

Data abstraction was performed by one of three reviewers into a standardised data extraction form and independently checked for accuracy by a second reviewer (AP, LU or MMJ). Discrepancies were resolved by discussion between the two reviewers and if agreement could not be reached, a third reviewer was consulted. Where multiple publications of the same study were identified, data were extracted and reported as a single study. Moreover, as this review of three rapid molecular tests incorporated an update of the most recent review of SeptiFast by Dark *et al*.,[50] all relevant data was extracted from the systematic review in the first instance, but were cross checked for accuracy with the original papers. When necessary, additional data was extracted from the original papers. For the review of SepsiTest and IRIDICA, all data was extracted from the original papers. Unpublished study data from the company (which was received during the review process) that met the inclusion criteria, were also extracted and quality assessed in accordance with the procedures outlined in this chapter.

The following information was extracted for all studies when reported: study characteristics (e.g. author, year of publication, country, study design, setting, funding); participant details (e.g. age, sex, inclusion and exclusion criteria); test details; reference standard details; and outcomes (including definitions).

2.1.4 Quality assessment strategy

The methodological quality of each included study was assessed by one of three reviewers and independently checked by a second reviewer (AP, LU or MMJ). Disagreements were resolved by discussion between the two reviewers and if agreement could not be reached, a third reviewer was consulted. The study quality characteristics were assessed according to (adapted) criteria based on those proposed by Whiting *et al.*, (QUADAS-2)[51] Further details are provided in Appendix 2.

2.1.5 Methods of data synthesis

The extracted data and quality assessment variables were presented for each study, both in structured tables and as a narrative description. The analysis comprised a narrative synthesis and pair-wise meta-analysis.

2.1.5.1 Meta-analysis

Where sufficient data existed, a meta-analysis was undertaken to generate pooled estimates of diagnostic parameters. The number of true positives, false negatives, false positives and true negatives from each study was meta-analysed to estimate sensitivity and specificity under the assumption that blood culture was 100% sensitive and specific. In brief, a bivariate normal model was used to model the population logit sensitivities and population logit specificities in each study to account for correlation between sensitivity and specificity within studies.[52] We assumed that the observed number of true positives in study i, TP_i, was binomially distributed with parameter, π_{Ai}, representing the study-specific sensitivity given the total number of positives on the reference test such that:

$$TP_i \sim Binomial\left(\pi_{Ai}, (TP_i + FN_i)\right)$$

Similarly, we assumed that the observed number of true negatives in study i, TN_i, was binomially distributed with parameter, π_{Bi}, representing the study-specific specificity given the total number of negatives on the reference test such that:

$$TN_i \sim Binomial\left(\pi_{Bi}, (FP_i + TN_i)\right)$$

We transform the parameters to the real line using the logit transformation such that:

$$\mu_{Ai} = logit(\pi_{Ai})$$
$$\mu_{Bi} = logit(\pi_{Bi})$$

Sensitivity and specificity are correlated within study such that higher values for sensitivity tend to be associated with lower values for specificity, and vice versa. We model this by assuming that the

study-specific logits for sensitivity and specificity arise from a bivariate normal distribution with population logits for sensitivity and specificity, $(\mu_A, \mu_B)^T$, respectively and variance-covariance matrix, Σ_{AB}, such that:

$$\begin{pmatrix}\mu_{Ai}\\ \mu_{Bi}\end{pmatrix} \sim N\left(\begin{pmatrix}\mu_A\\ \mu_B\end{pmatrix}, \Sigma_{\mathrm{AB}}\right)$$

$$\Sigma_{\mathrm{AB}} = \begin{pmatrix}\sigma_{\mathrm{A}}^2 & \sigma_{\mathrm{AB}}\\ \sigma_{\mathrm{AB}} & \sigma_{\mathrm{B}}^2\end{pmatrix}$$

σ_{A}^2 represents the variability in the logit sensitivities between studies, σ_{B}^2 represents the variability in the logit specificities between studies and σ_{AB} represents the covariance of the logit sensitivity and logit specificity.

The model was completed by giving the uncertain parameters the following prior distributions:

- $\mu_A \sim N(0, 1000)$
- $\mu_B \sim N(0, 1000)$
- $\Sigma_{\mathrm{AB}} \sim \mathrm{IW}\left(\begin{pmatrix}1 & 0\\ 0 & 1\end{pmatrix}, \upsilon = 2\right)$

  IW represents the inverse Wishart distribution on υ degrees of freedom.

This prior distribution has a between study standard deviation of 1.5 (95% CrI: 0.4, 32.4).
Where there were relatively few studies to estimate the variance-covariance matrix, Σ_{AB}, a weakly informative prior distribution was used such that:

- $\Sigma_{\mathrm{AB}} \sim \mathrm{IW}\left(\begin{pmatrix}1 & 0\\ 0 & 1\end{pmatrix}, \upsilon = 5\right)$

This prior distribution has a between study standard deviation of 0.5 (95% CrI: 0.3, 1.4).

Reasons for the heterogeneity in sensitivity and specificity between studies were explored using meta-regression. Models with and without covariates were compared using the deviance information criterion (DIC), which provides a relative measure of goodness-of-fit that penalises complexity and can be used to compare different models for the same likelihood and data.[53]

All parameters were estimated using Markov Chain Monte Carlo simulation implemented using the WinBUGS software package.[54] Analyses were conducted in R[55] using the R2WinBUGS interface package.[56] Convergence was assessed using the Gelman-Rubin convergence statistic. Convergence was achieved relatively quickly and generally within 5,000 iterations; in practice, a burn-in of 10,000 iterations was used. There was no evidence of high autocorrelation between successive samples of

the Markov chains. Results were displayed as forest plots and summary receiver operating curve (SROC) plots with 95% credible intervals (CrI) and 95% prediction intervals (PrI) for sensitivity and specificity.

2.1.5.2 Narrative synthesis

A meta-analysis was not conducted on a range of intermediate measures (i.e. time to result, time to treatment, test failure rates, duration of ICU and/or hospital stay, duration of broad and narrow spectrum antimicrobial therapy, re-admission rate and change in antimicrobial treatment plan) and clinical outcome measures (such as side-effects associated with broad spectrum antimicrobial use, morbidity and mortality, severity of disease, rates of superinfection, rates of resistant infections and health related quality of life) as the necessary data were not available or it was inappropriate to statistically pool studies because of their variability in reporting outcome data. Therefore, as suggested by the guidance produced by the Cochrane Collaboration[57] and the CRD for undertaking systematic reviews,[41,58] a narrative synthesis of included studies (grouped by outcome) was undertaken.

2.2 Clinical effectiveness results

2.2.1 Quantity and quality of research available

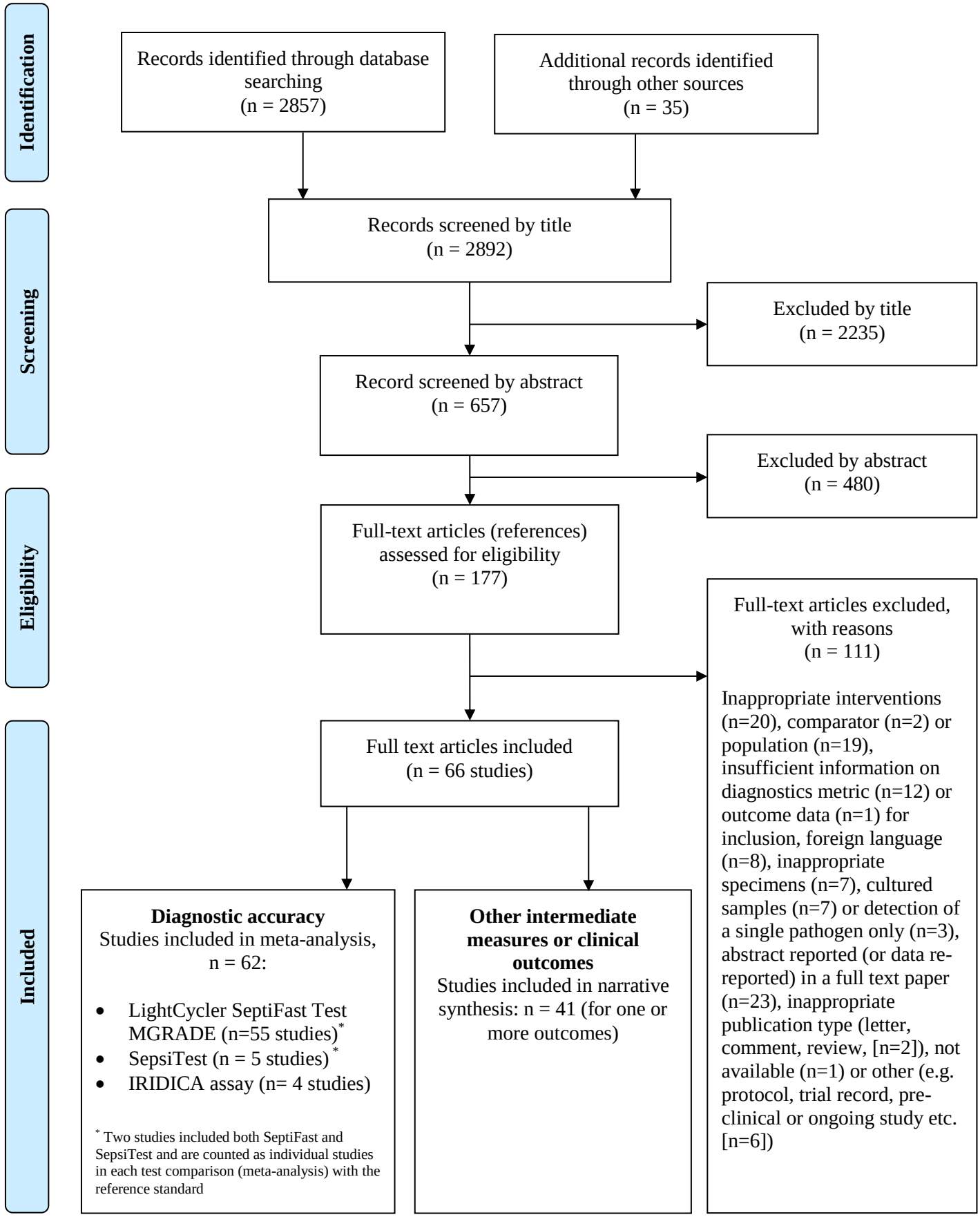
2.2.1.1 Number of studies identified/included

The literature searches identified 2892 citations. Of these, 66 studies met the inclusion criteria. A flow chart describing the process of identifying relevant literature can be found in Figure 2.

2.2.1.2 Number and type of studies excluded

A total of 111 full text articles were excluded as they did not me*et* all the pre-specified inclusion criteria. The majority of the articles were excluded primarily on the basis of having insufficient information to allow calculation of a diagnostic 2x2 metrics table (which includes data for true positives, false negatives, false positives and true negatives), incorrect population or interventions, or data reported in abstract form that were replaced by published full text papers. A full list of excluded studies with reasons for exclusion is presented in Appendix 3.

Figure 2: Study flow chart (adapted[59]): Clinical effectiveness review



2.2.2 Assessment of effectiveness

2.2.2.1 Description of included studies (design and patient characteristics)

- Study design characteristics

The design characteristics of the 66 included studies that evaluated the effectiveness of the SeptiFast, SepsiTest and IRIDICA in patients with suspected sepsis are summarised in Table 4 (further details are provided in Appendix 4).

In total, 56 single index test studies compared SeptiFast with blood culture,[45,46,60-113] and one study (Tafelski *et al*., 2015[114]) evaluated SeptiFast with blood culture and MALDI-TOF MS. All SeptiFast studies were single gate in design (i.e. same patient characteristics for both reference standard and index test). With the exception of three RCTs (Idelevich *et al.*, 2015;[112] Rodrigues *et al.*, 2013[102] and Tafelski *et al*., 2015[114]) all other SeptiFast studies were diagnostic cohort studies. Two single index test studies compared SepsiTest with blood culture (Nieman *et al*., unpublished;[115] Wellinghausen *et al*., 2009[48]) and one study evaluated SepsiTest with blood culture and MALDI-TOF MS (Loonen *et al*., 2014[116]). Two three-arm two index studies[117,118] compared both SeptiFast and SepsiTest with blood culture. Four single index test studies compared IRIDICA with blood culture,[49,119-121] two of which employed IRIDICA-PLEX-ID hybrid systems[49,121] (commercial-in-confidence data suggest that the IRIDICA CE certified systems is equivalent to the hybrid systems). All SepsiTest and IRIDICA studies were single gate diagnostic cohort studies.

Two SeptiFast studies (Louie *et al*., 2008;[46] Tsalik *et al*., 2010[82]) and one IRIDICA study (Bacconi *et al*., 2014;[49]) were conducted in North America. One IRIDICA study did not report the country (Delco-Volante *et al*., 2015[120]). Two SeptiFast studies were conducted in Brazil (Rodrigues *et al*., 2013;[102] Sitnik *et al*., 2014[109]), two were undertaken in Japan (Obara *et al*., 2011;[89] Yanagihara *et al*., 2010[84]), and one was undertaken in Turkey (Ozkaya-Parlakay *et al*., 2014[107]). Two SeptiFast studies were undertaken in the UK (Dark *et al*., 2009;[65] Warhurst *et al*., 2015[113]). [redacted]

Twenty-four of the SeptiFast studies (Avolio *et al*., 2014;[103] Bloos *et al*., 2010;[76] Gimeno *et al*., 2009;[67] Grif *et al*., 2012;[92] Hettwer *et al*., 2011;[86] Idelevich et al., 2015;[112] Josefson *et al*., 2011;[87] Lamoth *et al*., 2010;[77] Lehmann *et al*., 2010;[78] Lodes *et al*., 2009;[69] Louie *et al*., 2008;[46] Maubon *et al*., 2010;[79] Paolucci *et al*., 2013;[101] Rath *et al*., 2012;[97] Schaub *et al.* 2014;[108] Tafelski *et al.* 2015;[114] Wallet *et al*., 2010;[83] Yanagihara *et al*., 2010;[84] von Lilienfeld-Toal., *et al.* 2009;[73] Warhurst *et al*., 2015;[113] Sitnik *et al*., 2014;[109] Markota *et al*., 2014;[106] Ozkaya-Parlakay *et al*., 2014;[107] Rodrigues *et al*., 2013[102]), one multi-test SeptiFast and SepsiTest study (Schreiber *et al*., 2013;[118]), [redacted]

[redacted] had data collected prospectively.

Eight of the SeptiFast studies (Alvarez *et al.* 2012;[91] Calitri *et al.* 2015;[111] Dierkes *et al*., 2009;[66] Herne *et al*., 2013;[99] Lucignano *et al*., 2011;[88] Tschiedel *et al*., 2012;[98] Paolucci *et al*., 2009;[71] Lehmann *et al.* 2009[68]) and one SepsiTest study (Loonen *et al*., 2014[116]) had a retrospective design. One SeptiFast study evaluated samples collected both retrospectively and prospectively (Mancini *et al.*, 2014[105]). [redacted]

Where reported, the sampling period ranged from two months (Mancini *et al*., 2008[45]) to 66 months (Tsalik *et al*., 2010[82]).

The clinical setting (e.g., community, Emergency Department, in-hospital, intensive/critical care, general/specialist) was not reported for nine SeptiFast studies (Westh *et al*., 2009;[74] Raglio et a., 2006;[60] Vrioni *et al*., 2011;[90] Gimeno *et al.* 2009;[67] Idelevich et al. 2015;[112] Kasper et a., 2013;[100] Ozkaya-Parlakay et a., 2014;[107] Paolucci *et al*., 2009[71] Rodrigues *et al.* 2013[102]), one multi-test SeptiFast and SepsiTest study (Leitner *et al*., 2013[117]) [redacted] The setting across the remaining studies varied (Table 4). Explicit inclusion and exclusion criteria for patients were reported for seven SeptiFast studies (Bloos *et al.* 2010;[76] Bravo *et al.* 2011;[85] Idelevich et al. 2015;[112] Tsalik *et al*., 2010;[82] Schaub *et al.* 2014;[108] Warhurst *et al*., 2015;[113] Tafelski *et al*., 2015;[114]), [redacted]

With the exception of the SeptiFast study by Warhurst *et al*., (2015[113]), that reported on healthcare-associated bloodstream infections, and the SeptiFast study by Josefson *et al.*, (2011)[87] that reported on community-acquired bloodstream infections, reporting of whether infection was community or hospital acquired was unclear in the remainder of the included studies.

Table 4: Study characteristics of included studies

Author (year)	Country	Clinical Setting	Study design [a]	Total patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
SINGLE INDEX TEST STUDIES - SEPTIFAST						
Raglio *et al.* (2006)[60] (Abstract)	NR	NR	Single gate, NR	74 (114)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Bingold *et al.* (2007)[61] (Abstract)	Germany	Intensive/critical care	Single gate, NR	21 (134)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Klemm *et al.* (2007)[62] (Abstract)	Germany	Intensive/critical care	Single gate, NR	44 (56)	Test accuracy (patient), other intermediary/clinical outcomes	NR
Lodes *et al.* (2008)[63] (Abstract)	Germany	Intensive/critical care	Single gate, NR	137 (358)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Louie *et al.* (2008)[46]	USA	Emergency department, in hospital and intensive/ critical care	Single gate, Prospective	200 (200)	Test accuracy (patient), other intermediary/clinical outcomes	Roche Diagnostics
Mancini *et al.* (2008)[45]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	34 (103)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics
Vince *et al.* (2008)[64] (Correspondence)	Croatia	In hospital and intensive/ critical care	Single gate, NR	36 (39)	Test accuracy (sample)	NR
Dark *et al.* (2009)[65] (Correspondence)	UK	Intensive/critical care	Single gate, NR	50 (90)	Test accuracy (pathogen)	NR
Dierkes *et al.* (2009)[66]	Germany	Intensive/critical care	Single gate, Retrospective	77 (99)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics (partly)
Gimeno *et al.* (2009)[67] (Abstract)	Spain	NR	Single gate, Prospective	19 (45)	Test accuracy (sample)	NR
Lehmann *et al.* (2009)[68]	Germany	Emergency department, in hospital and intensive/ critical care	Single gate, Retrospective	436 (NR)	Intermediary/clinical outcomes	Roche Diagnostics (partly)

Author (year)	Country	Clinical Setting	Study design [a]	Total patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Lodes *et al.* (2009)[69]	Germany	Intensive/critical care	Single gate, Prospective	52 (258)	Test accuracy (sample)	NR
Palomares *et al.* (2009)[70] (Abstract)	Spain	Intensive/critical care	Single gate, NR	73 (76)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Paolucci *et al.* (2009)[71] (Correspondence)	Italy	NR	Single gate, Retrospective	34 (NR)	Test accuracy (patient), other intermediary/clinical outcomes	NR
Varani *et al.* (2009)[72]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	100 (130)	Febrile Test accuracy (episode)	NR
von Lilienfeld-Toal. *et al* (2009)[73]	Germany	In hospital	Single gate, Prospective	70 (784)	Test accuracy (pathogen)	Roche Diagnostics (partly)
Westh *et al.* (2009)[74]	Germany	NR	Single gate, NR	359 (558)	Test accuracy (pathogen), other intermediary/clinical outcomes	Roche Diagnostics
Berger *et al.* (2010)[75] (Abstract)	Austria	Neonatal unit	Single gate, NR	38 (38)	Test accuracy (patient)	NR
Bloos *et al.* (2010)[76]	Germany and France	Intensive/critical care	Single gate, Prospective	142 (236)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics
Lamoth *et al.* (2010)[77]	Switzerland	In hospital	Single gate, Prospective	86 (237)	Test accuracy (episode)	Roche Diagnostics
Lehmann *et al.* (2010)[78]	Germany	Intensive/critical care	Single gate, Prospective	108 (453)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics
Maubon *et al.* (2010)[79]	France	In hospital and unclear if intensive/critical care	Single gate, Prospective	110 (110)	Test accuracy (patient), other intermediary/clinical outcomes	Roche Diagnostics
Reguerio *et al.* (2010)[80]	Spain	In hospital and intensive/ critical care	Single gate, NR	72 (106)	Test accuracy (sample), other intermediary/clinical outcomes	No
Soki *et al.* (2010)[81] (Abstract)	Hungary	In hospital and intensive/ critical care	Single gate, NR	159 (162)	Test accuracy (sample)	NR

Author (year)	Country	Clinical Setting	Study design [a]	Total patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Tsalik *et al.* (2010)[82]	USA	Emergency department	Single gate, NR	306 (306)	Test accuracy (patient) other intermediary/clinical outcomes	No
Wallet *et al.* (2010)[83]	France	Intensive/critical care	Single gate, Prospective	72 (102)	Test accuracy (pathogen) other intermediary/clinical outcomes	Roche Diagnostics (partly)
Yanagihara *et al.* (2010)[84]	Japan	In hospital and Emergency department	Single gate, Prospective	212 (400)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics
Bravo *et al.* (2011)[85]	Spain	In hospital and intensive/ critical care	Single gate, NR	53 (53)	Test accuracy (episode)	NR
Hettwer *et al.* (2011)[86]	Germany	Emergency department	Single gate, Prospective	113 (113)	Test accuracy (patient)	Roche Diagnostics
Josefson *et al.* (2011)[87]	Sweden	In hospital	Single gate, Prospective	1093 (1141)	Test accuracy (patient), other intermediary/clinical outcomes	Roche Diagnostics (partly)
Lucignano *et al.* (2011)[88]	Italy	In hospital and intensive/ critical care	Single gate, Retrospective	803 (1553)	Test accuracy (sample)	NR
Obara *et al.* (2011)[89]	Japan	Emergency department, in hospital and intensive/ critical care	Single gate, NR	54 (78)	Test accuracy (sample)	Roche Diagnostics (Partly)
Vrioni *et al.* (2011)[90] (Abstract)	Greece	NR	Single gate, NR	33 (33)	Test accuracy (patient), other intermediary/clinical outcomes	NR
Alvarez *et al.* (2012)[91]	Spain	Intensive/critical care	Single gate, Retrospective	102 (NR)	Intermediary/clinical outcomes	NR
Grif *et al.* (2012)[92]	Austria	In hospital and intensive/ critical care	Single gate, Prospective	61 (71)	Test accuracy (sample), other intermediary/clinical outcomes	Pfizer
Guido *et al.* (2012)[93]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	166 (166)	Test accuracy (sample)	NR
Lodes *et al.* (2012)[94]	Germany	Intensive/critical care	Single gate, NR	104 (148)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Mauro *et al.* (2012)[95]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	79 (79)	Test accuracy (sample), other intermediary/clinical outcomes	NR

Author (year)	Country	Clinical Setting	Study design [a]	Total patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Pasquilani *et al.* (2012)[96]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	391 (391)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Rath *et al.* (2012)[97]	Germany	Intensive/critical care	Single gate, Prospective	170 (225)	Test accuracy (sample)	NR
Tschiedel *et al.* (2012)[98]	Germany	In hospital and intensive/ critical care	Single gate, Retrospective	75 (110)	Test accuracy (sample) other intermediary/clinical outcomes	NR
Herne *et al.* (2013)[99]	Estonia	In hospital and intensive/ critical care	Single gate, Retrospective	144 (160)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Kasper *et al.* (2013)[100]	Austria	NR	Single gate, NR	46 (NR)	Test accuracy (patient)	Roche Diagnostics (partly)
Paolucci *et al.* (2013)[101]	Italy	In hospital	Single gate, Prospective	201 (437)	Test accuracy (episode), other intermediary/clinical outcomes	No
Rodrigues *et al.* (2013)[102] (Abstract)	Brazil	NR	Single gate, Prospective RCT	46 (NR)	Intermediary/clinical outcomes	NR
Avolio *et al.* (2014)[103]	Italy	Emergency department and intensive/critical care	Single gate, Prospective	525 (525)	Test accuracy (pathogen), other intermediary/clinical outcomes	NR
Burdino *et al.* (2014)[104]	Italy	In hospital and intensive/ critical care	Single gate, NR	1024 (1186)	Test accuracy (sample)	NR
Mancini *et al.* (2014)[105]	Italy	In hospital	Single gate, Retrospective and prospective data	228 (NR)	Intermediary/clinical outcomes	Roche Diagnostics
Markota *et al.* (2014)[106]	Slovenia	Intensive/critical care	Single gate, Prospective	57 (63)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Ozkaya-Parlakay *et al.* (2014)[107]	Turkey	NR	Single gate, Prospective	69 (79)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Schaub *et al.* (2014)[108]	Switzerland	Emergency department	Single gate, Prospective	110 (205)	Test accuracy (patient), other intermediary/clinical outcomes	Roche Diagnostics (partly)

Author (year)	Country	Clinical Setting	Study design [a]	Total patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Sitnik *et al.* (2014)[109]	Brazil	Intensive/critical care (and oncology patients)	Single gate, Prospective	114 (114)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics (partly)
Barbanti *et al.* (2015)[110] (Abstract)	Italy	In hospital	Single gate, NR	491 (1837)	Test accuracy (sample)	NR
Calitri *et al.* (2015)[111]	Italy	In hospital and intensive/critical care	Single gate, retrospective	289 (NR)	Test accuracy (episode)	No
Idelevich *et al.* (2015)[112]	Germany	NR	Single gate, Prospective RCT	150 (253)	Test accuracy (pathogen), other intermediary/clinical outcomes	Roche Diagnostics and Pfizer (partly)
Tafelski *et al.* (2015)[114] [b]	Germany	Intensive/critical care	Single gate, Prospective RCT	78 (78)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Deutschland GmbH
Warhurst *et al.* (2015)[113]	UK	Intensive/critical care	Single gate, Prospective	795 (NR)	Test accuracy (pathogen) other intermediary/clinical outcomes	No
SINGLE INDEX TEST STUDIES - SEPSITEST						
Wellinghausen *et al*. (2009[48])	Germany	Intensive/critical care	Single gate, Prospective	187 (342)	Test accuracy (sample), other intermediary/clinical outcomes	No
Loonen *et al*. (2014)[116] b	Netherlands	Emergency department	Single gate, Retrospective	125 (NR)	Test accuracy (sample), other intermediary/clinical outcomes	Molzym GmbH (partly)
Nieman *et al.* (unpublished)[115]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
SINGLE INDEX TEST STUDIES - IRIDICA						
Bacconi *et al*. (2014)[49]	USA	Emergency department	Single gate, Prospective	331 (331)	Test accuracy (sample)	NR but majority authors are employees of Ibis Biosciences (an Abbott company)

Author (year)	Country	Clinical Setting	Study design [a]	Total patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Delco-Volante *et al*. (2015)[120] (conference presentation)	NR	NR	Single gate, Prospective	NR (81)	Test accuracy (sample)	Abbott
Vincent *et al*. (in press)[121]	Belgium, UK, Switzerland, France, Poland, Germany	Intensive/critical care	[redacted]	[redacted]	[redacted]	[redacted]
Metzgar *et al*. (unpublished)[119]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TWO INDEX TEST STUDIES – SEPTIFAST AND SEPSITEST						
Leitner *et al*. (2013)[117]	Austria	NR	Single gate, NR	57 (75)	Test accuracy (sample)	No
Schreiber *et al*. (2013)[118]	Germany	Intensive/critical care	Single gate, Prospective	50 (NR)	Test accuracy (patient), other intermediary/clinical outcomes	Molzym GmbH and Roche Diagnostics (partly)

[a] Single gate – a study design in which only patients with the target condition are recruited (i.e., single set of inclusion criteria for all participants, e.g., paired blood samples in sepsis patients; RCT comparing index test with reference standard in sepsis patients); double gate - a study design in which different sets of criteria are used for those with and those without the target condition (e.g., comparison between sepsis patients and healthy controls)

[b] Studies in which the reference standard was blood culture with MALDI-TOF MS plus clinical adjudication. All other studies used blood culture without MALDI-TOF MS plus clinical adjudication.

- **Patient characteristics of included studies**

The patient characteristics of the included studies are summarised in Table 5 (further details are provided in Appendix 4). Twenty-four of the SeptiFast studies (Avolio *et al.*, 2014;[103] Barbanti *et al.*, 2015;[110] Berger *et al.*, 2010;[75] Bingold *et al*., 2007[61] Burdino *et al.*, 2014;[104] Dark *et al.*, 2009;[65] Gimeno *et al.*, 2009[67] Hettwer *et al*., 2011;[86] Kasper *et al.*, 2013;[100] Klemm *et al*., 2007[62] Lodes *et al*., 2008;[63] Lucignano *et al.*, 2011;[88] Mauro *et al.*, 2012;[95] Palomares *et al.*, 2009;[70] Paolucci *et al.*, 2009[71] Paolucci *et al.*, 2013[101] Raglio *et al*., 2006[60] Soki *et al.*, 2010;[81] Varani *et al.*, 2009;[72] Vince *et al.*, 2008;[64] Vrioni *et al.*, 2011;[90] Wallet *et al.*, 2010;[83] Westh *et al*., 2009[74] Yanagihara *et al*., 2010[84]), one SepsiTest study (Wellinghausen *et al*., 2009[48]), one multi-test SeptiFast and SepsiTest study (Leitner *et al*., 2013[117]) [redacted] did not report on the mean or median age of patients. Six of the SeptiFast studies included both adults and children (Josefson *et al*., 2011;[87] Mauro *et al*., 2012;[95] Paolucci *et al.*, 2013;[101] Tschiedel *et al*., 2012;[98] Varani *et al.*, 2009;[72] Warhurst *et al*., 2015[113]), two included children and neonates (Calitri *et al.*, 2015;[111] Lucignano *et al.*, 2011[88]) and one included children and infants (Ozkaya-Parlakay *et al*., 2014[107]). Three SeptiFast studies (Berger *et al*., 2010;[75] Kasper *et al*., 2013;[100] Paolucci *et al*., 2009[71]) and one IRIDICA study (Delco-Volante *et al*., 2015[120]) included neonates and infants and one SepsiTest study included adults and children (Wellinghausen *et al*., 2009[48]). [redacted]

Twenty-two SeptiFast studies (Barbanti *et al*.2015;[110] Bravo *et al*., 2011;[85] Dark *et al*., 2009;[65] Gimeno *et al*., 2009;[67] Josefson *et al*., 2011;[87] Lamoth *et al*., 2010;[77] Mancini *et al*., 2008;[45] Reguerio *et al*., 2010;[80] Rodrigues *et al.*, 2013;[102] Tschiedel *et al*., 2012;[98] Varani *et al*., 2009;[72] Wallet *et al*., 2010;[83] Westh *et al*., 2009;[74] Palomares *et al*., 2009;[70] Paolucci *et al*., 2013;[101] Raglio *et al*., 2006;[60] Soki *et al*., 2010;[81] Vince *et al*., 2008;[64] von Lilienfeld-Toal., *et al*.2009;[73] Vrioni *et al*., 2011;[90] Kasper *et al*., 2013;[100] Ozkaya-Parlakay *et al*., 2014[107]) one multi-test SeptiFast and SepsiTest study (Leitner *et al*., 2013;[117]), [redacted] did not report details or a reference to a guideline for defining for sepsis. The remaining studies provided a description or a reference to a guideline for defining sepsis for included patients; however, these definitions and descriptions varied across studies and were sometimes not explicitly clear (Appendix 4).

Ten SeptiFast studies (Burdino *et al*., 2014;[104] Dierkes *et al*., 2009;[66] Louie *et al*., 2008;[46] Mancini *et al.*, 2008;[45] Mauro *et al*., 2012;[95] Pasquilani *et al*., 2012;[96] Varani *et al*., 2009;[72] Ozkaya-Parlakay *et al.* 2014;[107] Schaub *et al*., 2014;[108] Tafelski *et al*., 2015[114])

[redacted] reported on the proportion of the included patients who were immunocompromised. In addition, Paolucci *et al.* (2009)[71] reported that one patient was affected by primary congenital immunodeficiency; however, it was unclear if other immunocompromised patients were included in this study.

Twenty-three SeptiFast studies (Bravo *et al.*, 2011;[85] Burdino *et al.*, 2014;[104] Dierkes *et al.*, 2009;[66] Gimeno *et al.*, 2009;[67] Grif *et al.*, 2012;[92] Guido *et al.*, 2012;[93] Herne *et al.*, 2013;[99] Idelevich et al., 2015;[112] Kasper *et al.*, 2013;[100] Lodes *et al.*, 2012;[94] Markota *et al.*, 2014;[106] Maubon *et al.*, 2010;[79] Mauro *et al.*, 2012;[95] Palomares *et al.*, 2009;[70] Paolucci *et al.*, 2013;[101] Pasquilani *et al.*, 2012;[96] Rodrigues *et al.*, 2013;[102] Schaub *et al.*, 2014;[108] Tsalik *et al.*, 2010;[82] Varani *et al.*, 2009;[72] Vince *et al.*, 2008;[64] von Lilienfeld-Toal. *et al.*, 2009;[73] Warhurst *et al.*, 2015[113]) reported on the proportion of patients receiving antimicrobial therapy at the time of blood sampling. In addition, it was unclear in one SeptiFast study (Bloos *et al,.* 2010[76]) if patients received antimicrobial therapy (98%) prior to blood sampling. Of the 23 SeptiFast, six (Bravo *et al.*, 2011;[85] Guido *et al.*, 2012;[93] Kasper *et al.*, 2013;[100] Paolucci *et al.*, 2013;[101] Rodrigues *et al.*, 2013;[102] von Lilienfeld-Toal *et al*., 2009[73]) reported that none of the included patients had received antimicrobial therapy at the time of blood sampling. Similarly, in one IRIDICA study (Delco-Volante *et al.*, 2015[120]) none of the included patients received antimicrobial therapy at the time of blood sampling. In one multi-test SeptiFast and SepsiTest study (Schreiber *et al.*, 2013[118]) the majority of patients (72%) received antimicrobial therapy at recruitment. [redacted]

The SeptiFast studies by Alvarez *et al.*, (2012)[91] Bloos *et al*., (2010)[76] Bingold *et al*., (2007)[61] and Markota *et al*., (2014)[106] reported that all included participants had severe sepsis or septic shock. Bloos *et al*., (2010)[76] also reported a mean SOFA score of 10 and SAPS II score of 49 for the entire cohort. Markota *et al*., (2014)[106] reported a mean admission APACHE score for the cohort of 25 (±7.6 SD). The SeptiFast studies by Herne *et al*., (2013)[99] and Lehmann *et al*., (2010)[78] reported that all included patients had severe sepsis. Seven SeptiFast studies (Bravo *et al*., 2011;[85] Louie *et al*., 2008;[46] Maubon *et al*., 2010;[79] Rodrigues *et al.*, 2013;[102] Tafelski *et al,.* 2015[114]; Tsalik *et al*., 2010;[82] Schaub *et al*., 2014[108]) and the one multi-test SeptiFast and SepsiTest study (Schreiber *et al*., 2013[118]) reported mixed samples of patients with sepsis, severe sepsis and septic shock in varying proportions. The SeptiFast RCT by Rodrigues *et al.*, (2013)[102] reported a mean APACHE II of 17 for the SeptiFast group and 17 for the blood culture group, but was unclear whether this was at study entry or following testing. The SeptiFast RCT by Tafelski *et al*., (2015)[114] reported a median SAPS II on admission for the SeptiFast group of 40 (IQR 32-50) and the blood culture group of 47 (IQR 34-65). Schreiber *et al*., 2013[118] also reported a median SAPS II score of 41 (IQR 33 to 49) for the entire cohort.

[redacted]

Across the included studies, the number of patients analysed ranged from 19 (45 paired blood samples) (SeptiFast - Gimeno *et al.,* 2009[67]) to 1093 (1114 paired blood samples) (SeptiFast - Josefson *et al*., 2011[87]).

Table 5: Patient characteristics of included studies

Author (year)	Population	Mean [median] age, years	% Male	% Suspected community / hospital acquired infection	% Immuno-compromised patients	% Antibiotics at time of blood sample collection
SINGLE INDEX TEST STUDIES - SEPTIFAST						
Raglio *et al.* (2006)[60] (Abstract)	Patients with suspected sepsis	NR	NR	NR	NR	NR
Bingold *et al.* (2007)[61] (Abstract)	Surgical patients with severe sepsis and septic shock	NR	NR	NR	NR	NR
Klemm *et al.* (2007)[62] (Abstract)	Patients in intensive care with suspected sepsis	NR	NR	NR	NR	NR
Lodes *et al.* (2008)[63] (Abstract)	Surgical intensive care patients with suspected sepsis	NR	NR	NR	NR	NR
Louie *et al.* (2008)[46]	Adults with suspected sepsis	[46.5 average median]	61	NR	4	NR
Mancini *et al.* (2008)[45]	Adult neutropenic patients (with haematological malignancies) with suspected sepsis	47	67.6	NR	44.1	NR
Vince *et al.* (2008)[64] (Correspondence)	Patients with suspected sepsis	NR	NR	NR	NR	100
Dark *et al.* (2009)[65] (Correspondence)	Adults with suspected sepsis	NR	NR	NR	NR	NR
Dierkes *et al.* (2009)[66]	Adults with suspected sepsis	[55]	63.6	NR	45	79.2
Gimeno *et al.* (2009)[67]	Patients (oncohaematological) with febrile neutropenia	NR	NR	NR	NR	100
Lehmann *et al.* (2009)[68]	Adults with suspected sepsis	54.8	61.5	NR	NR	NR
Lodes *et al.* (2009)[69]	Adults with suspected sepsis	60.5	57.7	NR	NR	NR

Author (year)	Population	Mean [median] age, years	% Male	% Suspected community / hospital acquired infection	% Immuno-compromised patients	% Antibiotics at time of blood sample collection
Palomares *et al.* (2009)[70] (Abstract)	Adults with suspected sepsis	NR	NR	NR	NR	93.2
Paolucci *et al.* (2009)[71] (Correspondence)	Neonates with suspected sepsis	NR	NR	NR	NR	NR
Varani *et al.* (2009)[72]	Adults and children (immunocompromised) with suspected sepsis	NR	NR	NR	100	100
von Lilienfeld-Toal *et al.* (2009)[73]	Adults (haematological) with febrile neutropenia	[60]	54	NR	NR	0
Westh *et al.* (2009)[74]	Patients with suspected sepsis	NR	NR	NR	NR	NR
Berger *et al.* (2010)[75] (Abstract)	Very low birth weight infants (neonates) with suspected sepsis	NR	NR	NR	NR	NR
Bloos *et al.* (2010)[76]	Adults with severe sepsis or septic shock	66	68.5	NR	NR	95.8% on antibiotics (unclear if prior to blood sampling)
Lamoth *et al.* (2010)[77]	Adults (haematological) with febrile neutropenia	[54]	62	NR	NR	NR
Lehmann *et al.* (2010)[78]	Adults with suspected sepsis	58.4	66.7	NR	NR	NR
Maubon *et al.* (2010)[79]	Patients with malignancies and suspected sepsis	56.3	60.9	NR	NR	88.2
Reguerio *et al.* (2010)[80]	Adults with suspected sepsis	64	73.6	NR	NR	NR

Author (year)	Population	Mean [median] age, years	% Male	% Suspected community / hospital acquired infection	% Immuno-compromised patients	% Antibiotics at time of blood sample collection
Soki *et al.* (2010)[81] (Abstract)	Septic patients in intensive care or with haematological malignancies	NR	NR	NR	NR	NR
Tsalik *et al.* (2010)[82]	Adults with suspected sepsis	54.1	54.9	NR	NR	22.5
Wallet *et al.* (2010)[83]	Adults with suspected sepsis	NR	NR	NR	NR	NR
Yanagihara *et al.* (2010)[84]	Patients with suspected sepsis	NR	64.6	NR	NR	NR
Bravo *et al.* (2011)[85]	Adult ICU patients who were critically ill with suspected sepsis	[65.5]	62.3	NR	NR	0
Hettwer *et al.* (2011)[86]	Adults with suspected sepsis	NR	NR	NR	NR	NR
Josefson *et al.* (2011)[87]	Adults and children with suspected sepsis	[67]	55.5	100 (community acquired)	NR	NR
Lucignano *et al.* (2011)[88]	Neonates and children with suspected sepsis	NR	NR	NR	NR	NR
Obara *et al.* (2011)[89]	Adults with suspected sepsis	61.6	64.8	NR	NR	NR
Vrioni *et al.* (2011)[90] (Abstract)	Patients with suspected sepsis	NR	NR	NR	NR	NR
Alvarez *et al.* (2012)[91]	Adults with severe sepsis or septic shock	64.9	78.4	NR	NR	NR
Grif *et al.* (2012)[92]	Adults with suspected sepsis	55.6	68.9	NR	NR	91.8
Guido *et al.* (2012)[93]	Adult neutropenic patients (with haematological malignancies) with suspected sepsis	[66.1]	62	NR	NR	0
Lodes *et al.* (2012)[94]	Adults with suspected sepsis	63.1	71.1	NR	NR	79.7% of samples under antibiotic therapy and 41.9% under antifungal.

Author (year)	Population	Mean [median] age, years	% Male	% Suspected community / hospital acquired infection	% Immuno-compromised patients	% Antibiotics at time of blood sample collection
Mauro *et al.* (2012)[95]	Adults and children, immunocompromised with suspected sepsis	NR	51.9	NR	100	5
Pasquilani *et al.* (2012)[96]	Adults with suspected sepsis	[73]	55	NR	4.3	48.8
Rath *et al.* (2012)[97]	Adults (who have undergone liver transplantation or other major abdominal surgery) with suspected sepsis	56.4	56.5	NR	NR	NR
Tschiedel *et al.* (2012)[98]	Adults and children with suspected sepsis	[6]	49.3	NR	NR	NR
Herne *et al.* (2013)[99]	Adults with suspected sepsis	58	42.4	NR	NR	99.3
Kasper *et al.* (2013)[100]	Very low birth weight premature infants with suspected sepsis	NR	NR	NR	NR	0
Paolucci *et al.* (2013)[101]	Adults and children (haematological) with severe febrile neutropenia	NR	NR	NR	NR	0
Rodrigues *et al.* (2013)[102] (Abstract)	Adults with suspected sepsis	64.5	67.4	NR	NR	0
Avolio *et al.* (2014)[103]	Adults with suspected sepsis	NR	NR	NR	NR	NR
Burdino *et al.* (2014)[104]	Adults with suspected sepsis	NR	NR	NR	10.5%	89
Mancini *et al.* (2014)[105]	Adults (haematological patients) with suspected sepsis	48.6	66.7	NR	NR	NR
Markota *et al.* (2014)[106]	Adults with severe sepsis or septic shock	59.5	66.7	NR	NR	61.9
Ozkaya-Parlakay *et al.* (2014)[107]	Children with severe sepsis or septic shock	2.7	62.3	NR	0	NR
Schaub *et al.* (2014)[108]	Adults with suspected sepsis	[64]	51	NR	13.6	14.5

Author (year)	Population	Mean [median] age, years	% Male	% Suspected community / hospital acquired infection	% Immuno-compromised patients	% Antibiotics at time of blood sample collection
Sitnik *et al.* (2014)[109]	Adults with suspected sepsis	49.7	64.9	NR	NR	NR
Barbanti *et al.* (2015)[110]	Patients (haematological) with febrile neutropenia and suspected sepsis	NR	NR	NR	NR	NR
Calitri *et al.* (2015)[111]	Children and neonates with with suspected sepsis, febrile neutropenia, fever without focus or localised infective focus	[6.8]	63.3	NR	NR	NR
Idelevich *et al.* (2015)[112]	Adults with febrile neutropenia or afebrile neutropenia with sepsis	52.4	59.3	NR	NR	100
Tafelski *et al.* (2015)[114]	Adults with suspected sepsis	[63, average median]	64.1	NR	15.4	NR
Warhurst *et al.* (2015)[113]	Adults (and children over 16 years) with suspected sepsis	[58]	60	100 (healthcare acquired)	NR	85.7
SINGLE INDEX TEST STUDIES - SEPSITEST						
Wellinghausen *et al.* (2009)[48]	Adults and children with SIRS, sepsis (79.1%) or haematological patients with neutropenic fever (20.9%)	NR	NR	NR	NR	NR
Loonen *et al.* (2014)[116]	Adults with suspected sepsis	64.7	59.2	NR	NR	NR
Nieman *et al.* (unpublished)[115]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
SINGLE INDEX TEST STUDIES - IRIDICA						
Bacconi *et al.* (2014)[49]	Adults with suspected sepsis	NR	NR	NR	NR	NR

Author (year)	Population	Mean [median] age, years	% Male	% Suspected community / hospital acquired infection	% Immuno-compromised patients	% Antibiotics at time of blood sample collection
Delco-Volante *et al.* (2015)[120] (conference presentation)	Neonates with suspected sepsis	[0]	NR	NR	NR	0
Vincent *et al.* (in press)[121]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Metzgar *et al.* (unpublished)[119]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TWO INDEX TEST STUDIES – SEPTIFAST AND SEPSITEST						
Leitner *et al.* (2013)[117]	Critically ill patients with suspected sepsis	NR	NR	NR	NR	NR
Schreiber *et al.* (2013)[118]	Adults with suspected sepsis	[64]	80	NR	NR	72

- **Details of index and reference tests, blood sampling methods and CE approval**

A detailed summary of the index and reference tests, blood samples taken and interval between the index and reference test, CE approval of the blood volume used for testing, definition of a true positive, laboratory working times and the unit of analysis (pathogen/sample/patient/episode) is presented in Appendix 4.

Thirty-four of the SeptiFast studies reported on the blood volume used for the SeptiFast test.[45,71,73-80,82-85,87-89,92-96,98-101,103-105,109,111-114,116] Of these, nine studies reported blood volumes that did not comply with CE approval: Lehmann *et al*., (2010)[78] Lodes *et al*., (2012)[94] and von Lilienfeld-Toal *et al*., (2009)[73] all reported using 1 ml in adults; Bloos *et al*., (2010)[76] Lamoth *et al.*, (2010)[77] Paolucci *et al.*, (2013)[101] and Sitnik *et al*., (2014)[109] all reported using 3 ml in adults; Berger *et al*., (2010)[75] reported using 0.1 ml in neonates and infants, and Kasper *et al*., (2013)[100] reported using 0.1 to 0.7 ml in neonates and infants. The remainder of the SeptiFast studies did not report the blood volume used for the test.

Thirty-eight SeptiFast studies reported that blood drawn for SeptiFast and for blood culture were drawn at the same time.[45,62,66,67,70,72-74,76-78,80,83-89,92-101,103,104,106-109,112-114] Of these, one SeptiFast study reported that blood drawn for SeptiFast and for blood culture were drawn within one hour (Burdino *et al*., 2014[104]) and another study reported that blood drawn for SeptiFast and for blood culture were drawn within 12 hours of each other (Herne *et al*., 2013[99]). The remainder of the SeptiFast studies did not report on when blood samples were drawn.

Across the studies evaluating SeptiFast, the studies by Lehmann *et al.*, (2009)[68] Westh *et al*., (2009)[74] Tsalik *et al.*, (2010)[82] Wallet *et al.*, (2010)[83] and Yanagihara *et al*., (2010)[84] all reported that either the BACTEC or BacT/ALERT blood systems were used and was dependant on the testing site performing the assay. Across the remaining SeptiFast studies, nineteen reported using the BACTEC system (Bravo *et al.*, 2011;[85] Dierkes *et al.*, 2009;[66] Idelevich *et al.*, 2015;[112] Josefson *et al.*, 2011;[87] Klemm *et al.*, 2007;[62] Lamoth *et al.*, 2010;[77] Lehmann *et al.*, 2010;[78] Lodes *et al.*, 2012;[94] Lucignano *et al.*, 2011;[88] Mauro *et al.*, 2012;[95] Obara *et al.*, 2011;[89] Pasquilani *et al.*, 2012;[96] Rath *et al.*, 2012;[97] Sitnik *et al.*, 2014;[109] Soki *et al.*, 2010;[81] Tafelski *et al.*, 2015;[114] Tschiedel *et al.*, 2012;[98] Varani *et al.*, 2009;[72] von Lilienfeld-Toal., *et al.*, 2009[73]). Of these, one used the BACTEC system with MALDI-TOF MS (Tafelski *et al*., 2015[114]). Seventeen studies reported using the BacT/ALERT system (Avolio *et al*., 2014;[103] Barbanti *et al*., 2015;[110] Burdino *et al*., 2014;[104] Calitri *et al*., 2015;[111] Grif *et al*., 2012;[92] Guido *et al*., 2012;[93] Herne *et al*., 2013;[99] Hettwer *et al*., 2011;[86] Kasper *et al*., 2013;[100] Mancini *et al*., 2008;[45] Mancini *et al.*, 2014;[105] Markota *et al.*, 2014;[106] Paolucci *et al*., 2013;[101] Reguerio *et al.* 2010;[80] Rodrigues *et al.*, 2013;[102] Schaub *et al*., 2014;[108] Warhurst *et al*., 2015[113]). The remaining studies did not report the method used.

Details of the laboratory working times or when assays were carried out for the index test were reported by thirteen studies evaluating SeptiFast (Avolio *et al*., 2014;[103] Burdino *et al*., 2014;[104] Dierkes *et al*., 2009;[66] Grif *et al*., 2012;[92] Herne *et al*., 2013;[99] Idelevich et al., 2015;[112] Lehmann *et al*., 2010;[78] Mancini *et al*., 2008;[45] Mancini *et al.*, 2014;[105] Markota *et al*., 2014;[106] Paolucci *et al.*, 2013;[101] Tschiedel *et al*., 2012;[98] Tafelski *et al*., 2015[114]). Working times were seven days per week for four SeptiFast studies (Avolio *et al*., 2014;[103] Dierkes *et al*., 2009;[66] Herne *et al*., 2013;[99] Mancini *et al*., 2008[45]), six days per week for one SeptiFast study (Markota *et al*., 2014[106]) and five days per week for six SeptiFast studies (Grif *et al.*, 2012;[92] Idelevich et al., 2015;[112] Mancini *et al.*, 2014;[105] Paolucci *et al.*, 2013;[101] Tafelski *et al*., 2015[114] Tschiedel *et al.*, 2012[98]). For the remainder of the studies reporting on working times, it was unclear how many days of the week laboratories were working.

Definition of a true positive was reported by twelve SeptiFast studies (Avolio *et al*., 2014;[103] Bloos *et al*., 2010;[76] Bravo *et al*., 2011;[85] Herne *et al*., 2013;[99] Josefson *et al*., 2011;[87] Lehmann *et al*., 2010;[78] Leitner *et al*., 2013;[117,123] Lucignano *et al*., 2011;[88] Pasquilani *et al*., 2012;[96] Schaub *et al*., 2014;[108] Schreiber *et al*., 2013;[118] Tafelski *et al*., 2015[114]). Definition of a true positive varied across these studies (see Table 6).

A range of metrics (units of analyses) were used to assess the diagnostic accuracy of SeptiFast. In eleven studies the unit of analyses was at the 'patient level' (Hettwer *et al*., 2011;[86] Berger *et al*., 2010;[75] Klemm *et al*., 2007;[62] Josefson *et al*., 2011;[87] Louie *et al*., 2008;[46] Maubon *et al*., 2010;[79] Tsalik *et al*., 2010;[82] Vrioni *et al*., 2011;[90] Kasper *et al*., 2013;[100] Schaub *et al*., 2014;[108] Paolucci *et al*., (2009[71]), pathogen level in seven studies (Avolio *et al*., 2014;[103] Dark *et al*., 2009;[65] Idelevich et al., 2015;[112] von Lilienfeld-Toal., *et al*., 2009;[73] Wallet *et al*., 2010;[83] Warhurst *et al*., 2015;[113] Westh *et al*., 2009;[74]) and episodes for five studies (Bravo *et al*., 2011;[85] Calitri *et al.*, 2015;[111] Lamoth *et al.*, 2010;[77] Paolucci *et al.*, 2013[101] Varani *et al*., 2009[72]). For the remainder of the studies evaluating SeptiFast against blood culture (with or without blood culture) the unit of analysis was samples. Whilst the heterogeneity in the metrics has the potential to introduce some bias, the impact on the results was believed to be modest.

Thirty studies evaluating SeptiFast against blood culture included contaminants in the diagnostic test accuracy analysis in this assessment report.[45,60,66,69-72,75,78,80,83-87,89,93-100,107-109,111,112,122] and eight studies reported that contaminants were excluded.[46,74,82,88,101,103,104,113] For the remainder of the SeptiFast studies it was unclear if contaminants were included or excluded.

[redacted]

One study did not report when bloods were drawn (Loonen *et al*., 2014[116]). One study performed blood culture using a BACTEC system (Wellinghausen *et al*., 2009[48]), [redacted] and one study reported using BacT/ALERT with MALDI-TOF MS (Loonen *et al*., 2014[116]). [redacted] Definition of a true positive was reported by one SepsiTest study (Wellinghausen *et al*., 2009[48]). [redacted]

[redacted]

). The study by Delco-Volante *et al*., (2015)[120] reported using 0.5 ml in neonates and infants. [redacted]

Both of the studies evaluating both SeptiFast and SepsiTest against blood culture did not report the volume of blood used for the index test assay (Leitner *et al*., 2013;[117] Schreiber *et al*., 2013[118]). Leitner *et al*., (2013)[117] reported that the reference standard and index tests were performed on blood samples drawn at the same time. Schreiber *et al*., (2013)[118] did not report if blood samples for the

index tests and blood culture assay were drawn at the same time. Both studies reported that blood culture was undertaken using the BACTEC system. Both studies reported a definition of a true positive. Neither study reported on laboratory working times. The unit of analysis for the study by Leitner *et al.,* (2013)[117] was samples and the unit of analysis for Schreiber *et al.,* (2013)[118] was patients. Both studies included contaminants in the diagnostic test accuracy analysis.

2.2.2.2 Quality characteristics

The QUADAS-2 tool,[51] designed to evaluate the methodological quality of diagnostic accuracy studies, includes four key domains relating to patient selection, index test, reference standard and flow and timing. Using a set of signalling questions, each domain is assessed in terms of risk of bias (low, high or unclear risk [in the event of insufficient data in the publication to answer the corresponding question]) and the first three domains are also assessed in terms of applicability (no, yes or unclear concerns).

The overall methodological quality of the 66 included studies is summarised in Figure 3 and Table 7. The methodological quality of the included studies, as assessed using the QUADAS-2 tool, was variable.

[redacted]

Figure 3: **Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies**

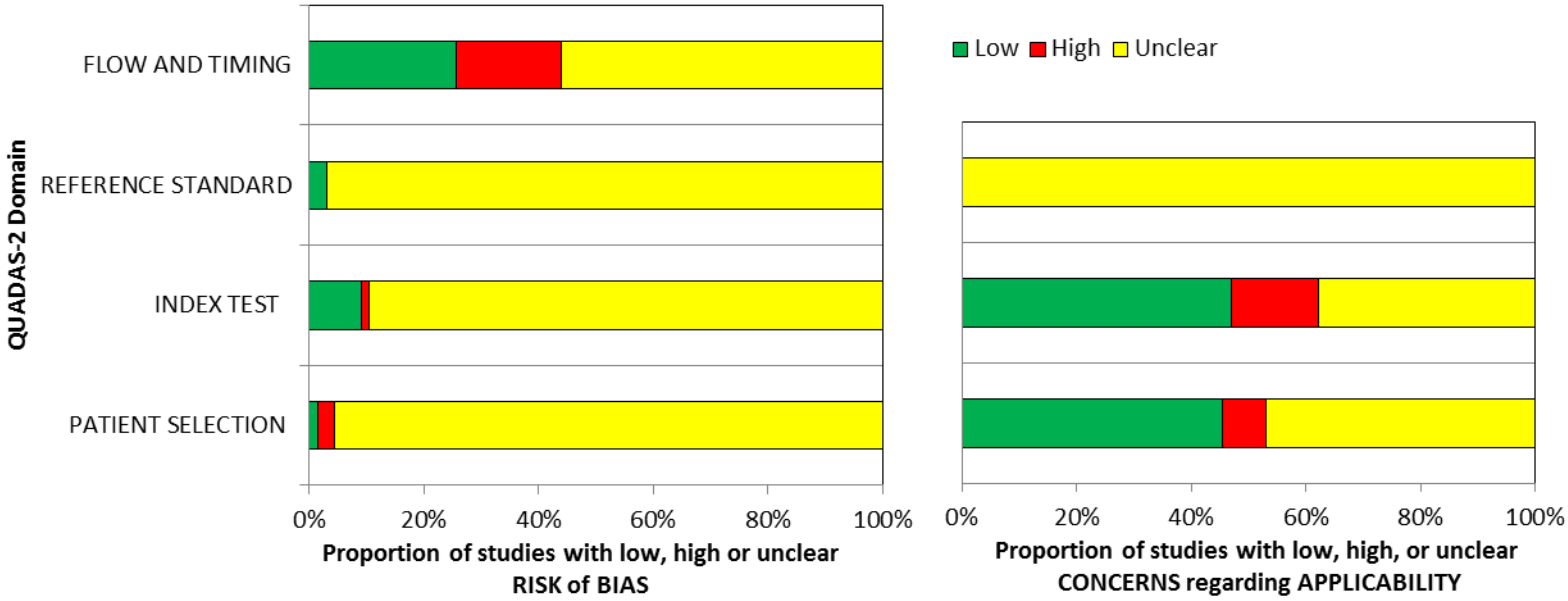


Table 6: Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Raglio *et al*. (2006)[60] (abstract)	U	U	U	U	U	U	U
Bingold *et al*. (2007)[61] (abstract)	U	U	U	U	U	U	U
Klemm *et al*. (2007)[62] (abstract)	U	U	U	U	U	U	U
Lodes *et al*. (2008)[63] (abstract)	U	U	U	U	U	U	U
Louie *et al*. (2008)[46]	U	U	U	U	N	U	U
Mancini *et al*. (2008)[45]	U	U	U	L	N	N	U
Vince *et al*. (2008)[64] (correspondence)	U	U	U	U	U	U	U
Dark *et al*. (2009)[65] (correspondence)	U	L	U	U	N	U	U
Dierkes *et al*. (2009)[66]	U	U	U	U	Y	U	U
Gimeno *et al*. (2009)[67] (abstract)	U	U	U	U	U	U	U
Lehmann *et al*. (2009)[68]	U	U	U	H	N	U	U
Lodes *et al*. (2009)[69]	U	U	U	U	U	U	U
Palomares *et al*. (2009)[70] (abstract)	U	U	U	U	U	U	U
Paolucci *et al*. (2009)[71] (correspondence)	U	U	U	U	N	N	U
Varani *et al*. (2009)[72]	U	U	U	L	N	U	U
Von Lilienfeld-Toal *et al*. (2009)[73]	U	U	U	U	U	Y	U
Wellinghausen *et al*. (2009)[48]	U	U	U	L	U	N	U
Westh *et al*. (2009)[74]	U	U	U	H	U	N	U
Berger *et al*. (2010)[75] (abstract)	U	U	U	U	U	Y	U
Bloos *et al*. (2010)[76]	U	L	U	U	N	Y	U
Lamoth *et al*. (2010)[77]	U	U	U	U	U	Y	U
Lehmann *et al*. (2010)[78]	U	U	U	L	N	Y	U
Maubon *et al*. (2010)[79]	U	U	U	U	N	N	U
Reguerio *et al*. (2010)[80]	U	U	U	L	N	N	U

Soki *et al*. (2010)[81] (abstract)	U	U	U	U	U	U	U
Tsalik *et al*. (2010)[82]	U	U	U	H	N	N	U
Wallet *et al*. (2010)[83]	U	U	U	H	U	N	U
Yanagihara *et al*. (2010)[84]	U	U	U	H	N	N	U
Bravo *et al*. (2011)[85]	H	L	U	U	Y	N	U
Hettwer *et al*. (2011)[86]	U	U	U	H	N	U	U
Josefson *et al*. (2011)[87]	U	U	U	H	U	N	U
Lucignano *et al*. (2011)[88]	U	U	U	H	U	N	U
Obara *et al*. (2011)[89]	U	U	U	L	N	N	U
Vrioni *et al*. (2011)[90] (abstract)	U	U	U	U	U	U	U
Alvarez *et al*. (2012)[91]	U	H	U	U	N	U	U
Grif *et al*. (2012)[92]	U	U	U	L	N	N	U
Guido *et al*. (2012)[93]	U	U	U	L	U	N	U
Lodes *et al*. (2012)[94]	U	U	U	L	N	Y	U
Mauro *et al*. (2012)[95]	U	U	U	L	N	N	U
Pasquilani *et al*. (2012)[96]	U	U	U	L	U	N	U
Rath *et al*. (2012)[97]	U	U	U	L	Y	U	U
Tschiedel *et al*. (2012)[98]	U	U	U	L	U	N	U
Herne *et al*. (2013)[99]	U	U	U	H	N	N	U
Kasper *et al*. (2013)[100]	U	U	U	L	U	Y	U
Leitner *et al*. (2013)[117]	U	U	U	U	U	U	U
Paolucci *et al*. (2013)[101]	U	U	U	U	U	Y	U
Rodrigues *et al*. (2013)[102] (abstract)	U	U	U	U	U	U	U
Schreiber *et al*. (2013)[118]	U	L	U	U	N	U	U
Avolio *et al*. (2014)[103]	U	U	U	H	Y	N	U
Bacconi *et al*. (2014)[49]	U	U	U	U	U	N	U
Burdino *et al*. (2014)[104]	U	U	U	L	U	N	U
Loonen *et al*. (2014)[116]	U	U	U	H	U	N	U
Mancini *et al*. (2014)[105]	U	U	U	U	N	N	U

Markota *et al*. (2014)[106]	U	U	U	U	N	U	U
Ozkaya-Parlakay *et al*. (2014)[107]	U	U	U	U	N	U	U
Schaub *et al*. (2014)[108]	U	U	U	U	N	U	U
Sitnik *et al*. (2014)[109]	U	U	U	U	N	Y	U
Barbanti *et al.* (2015)[110]	U	U	U	U	U	U	U
Calitri *et al.* (2015)[111]	U	U	U	U	Y	N	U
Delco-Volante *et al*. (2015)[120] (conference presentation)	U	U	U	U	U	Y	U
Idelevich *et al.* (2015) [112]	U	U	U	U	U	N	U
Tafelski *et al*. (2015)[114]	H	L	L	L	N	N	U
Warhurst *et al*. (2015)[113]	L	L	L	L	N	N	U
[redacted]							

L or N, low risk of bias or having low concerns regarding applicability; H or Y, High risk of bias or having concerns regarding applicability; U, unclear risk of bias or having concerns regarding applicability

2.2.2.3 Effectiveness of the interventions

This section presents the results of the following separately

- An assessment of diagnostic test accuracy (meta-analysis, where applicable) of each diagnostic tests (i.e. SeptiFast, SepsiTest and IRIDICA in conjunction with clinical assessment) for rapidly identifying bloodstream bacteria and fungi.
- An assessment of each diagnostic test on a range of other intermediate and clinical outcome measures (narrative synthesis).

Analyses were undertaken to assess the sensitivity of the results to alternative priors but these made little difference and thus only the results using the priors detailed in Section 2.1.5.1 have been presented.

2.2.2.3.1 Diagnostic test accuracy

A total of 62 studies contributed to the meta-analysis of sensitivity and specificity including two studies (Leitner *et al.*, 2013[117] and Schreiber *et al.*, 2013[118]) which were 3-arm (two index tests) studies. For simplicity, the correlation between tests was ignored in the analyses.

In total, 54 studies evaluated SeptiFast compared with blood culture, four studies evaluated SepsiTest compared with blood culture and four studies evaluated IRIDICA compared with blood culture. Separate meta-analyses are presented for each of these three tests in Sections 2.3.1.1 to 2.3.1.3. In addition, one study (Tafelski *et al*., 2015)[114] evaluated SeptiFast compared with blood culture plus MALDI-TOF MS and one study (Loonen *et al*., 2014)[116] evaluated SepsiTest compared with blood culture plus MALDI-TOF MS. Since there was only one study for each of these comparisons, no meta-analysis was conducted and the data were summarised narratively.

2.2.2.3.1.1 SeptiFast test

2.2.2.3.1.1.1 SeptiFast test compared with blood culture

The pooled sensitivity and specificity of SeptiFast compared with blood culture (n=54 studies) were 0.65 (95% CrI: 0.60, 0.71) and 0.86 (95% CrI: 0.84, 0.89), respectively (Figure 4). The 95% prediction intervals of 0.29, 0.90 (sensitivity) and 0.62, 0.96 (specificity) suggest considerable uncertainty in predicting the sensitivity and specificity of a new study. The between-study standard deviations for logit sensitivity and specificity were estimated to be 0.76 (95% CrI: 0.57, 1.01) and 0.66 (95% CrI: 0.53, 0.85), with correlation -0.05 (95% CrI: -0.38, 0.28). Figure 5 presents the joint distribution for sensitivity and specificity and highlights the extent of the heterogeneity between studies (as indicated by the 95% prediction interval). The black circles represent the sensitivity and specificity estimates from each study, with the size reflecting the study sample size. The proportion of

discordant results with blood culture (i.e. cases of disagreement between the reference standard and the index test) varied across studies from 6% to 46%, with median 17%.

Figure 4: Sensitivity and specificity of SeptiFast compared with blood culture

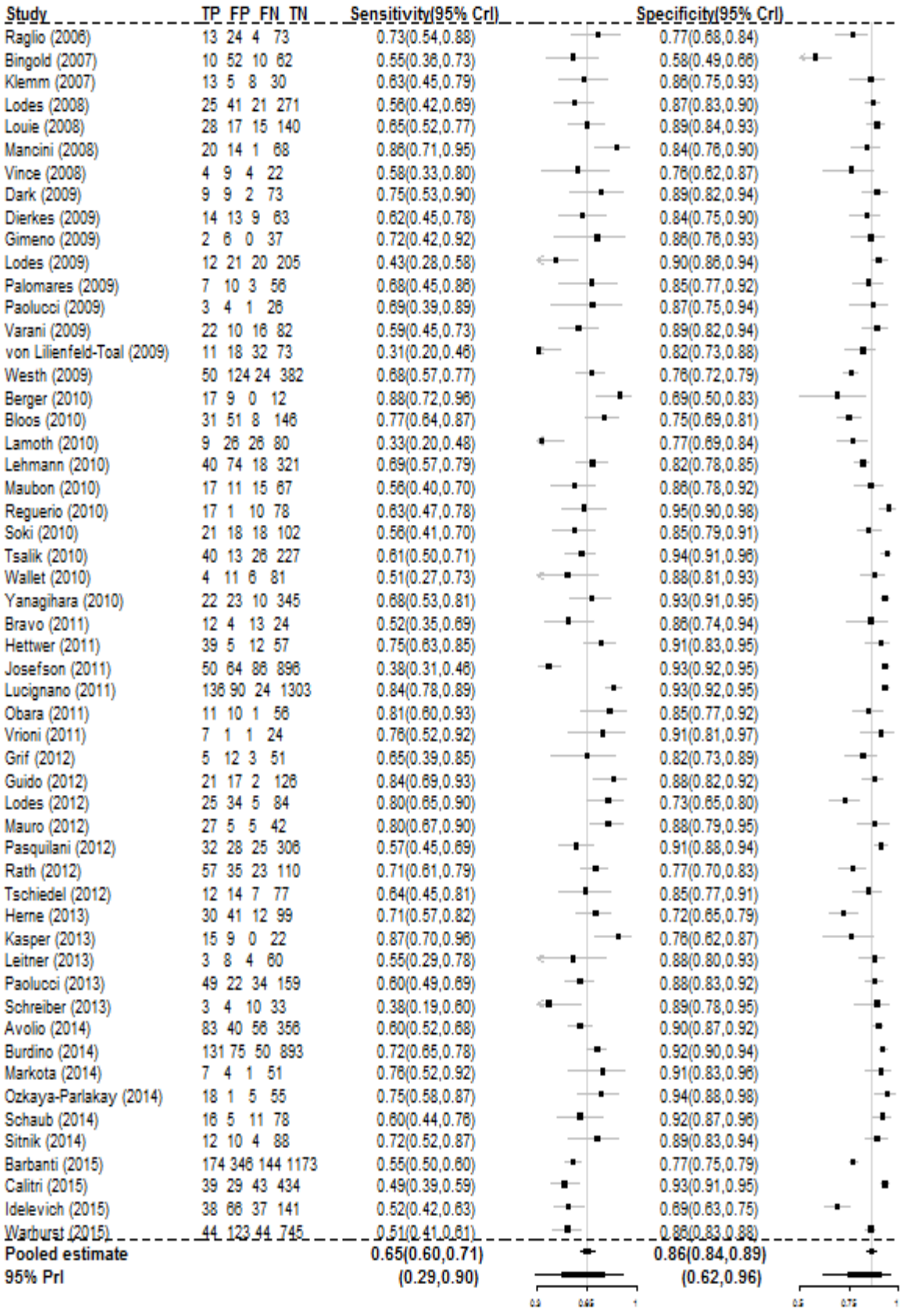
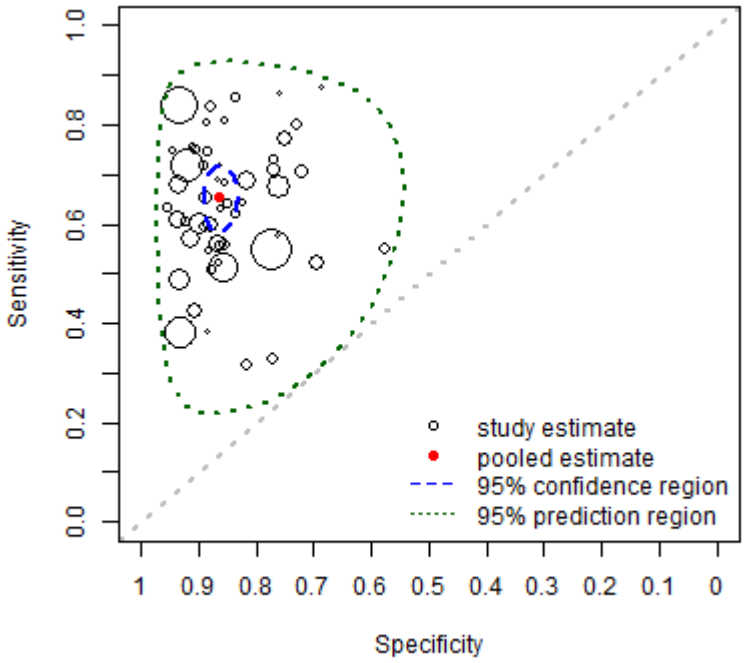


Figure 5: Summary receiver operating curve plot of SeptiFast compared with blood culture (All Studies)



Additional analyses were undertaken for the following subgroups only: neonates and children, antibiotic use prior to blood sample collection, suspected community or health acquired infection, patients with febrile neutropenia and studies that included/excluded contaminants in the data analysis. There was insufficient information on studies at low risk of bias (see Section 2.2.2.2) and people who were immunocompromised to allow a meaningful estimate of test accuracy.

- Neonates and children

Six studies provided data on children and neonates. Of these, three studies included neonates only (Paolucci *et al.*, 2009;[71] Berger *et al.*, 2010[75] and Kasper *et al.*, 2013),[100] one included children only aged 1 month to 17 years (Ozkaya-Parlakay *et al.*, 2014)[107] and two included both neonates and children (Lucignano *et al.*, 2011[88] and Calitri *et al.*, 2015[111]). Of the remaining studies, six were conducted in adults and children, 28 in adults, and 14 did not report the age of participants. Based on comparison of models with and without covariates for an age category, there was no evidence that sensitivity and specificity was affected by the age of the subjects (Appendix 5).

- People exposed to antibiotics prior to blood sample collection

The proportion of patients receiving antibiotics prior to blood draw was recorded in 24 studies and ranged from 0% to 100% with median 72%. The remaining studies either did not report prior exposure to antibiotics, or provided only limited information and were therefore excluded from the

analysis. There was no evidence that exposure to antibiotics prior to blood sample collection affected the estimates of sensitivity and specificity (Appendix 5).

- People with suspected community or health acquired infection

Clinical setting was used as a proxy for suspected community or health acquired infection. Studies were grouped according to whether infection was diagnosed in hospital (38 studies), emergency department (three studies), mixed setting of emergency or other hospital department (four studies) or not recorded (nine studies). Based on comparison of models with and without covariates for the clinical setting, there was no evidence that this affected sensitivity and specificity (Appendix 5).

- People with febrile neutropenia

In total, eight studies provided data on patients with febrile neutropenia. Of these, six studies included patients (100%) with febrile neutropenia only (Gimeno *et al.*, 2009;[67] Von Lilienfeld-Toal *et al.*, 2009;[73] Lamoth *et al.*, 2010;[77] Guido *et al.*, 2012;[93] Paolucci *et al.*, 2013[101] and Barbanti *et al.* 2015.[110] Studies by Mancini *et al.* (2008)[45] and Idelevich *et al.* (2015)[112] reported that 92% and 98% of patients had febrile neutropenia, respectively. Based on comparison of models with and without covariates for the presence of patients with febrile neutropenia, there was no evidence that this affected sensitivity and specificity (Appendix 5).

- Studies with inclusion/exclusion of contaminants

In total, 32 studies included contaminants in the reported results, eight studies excluded contaminants and 14 did not report on handling of contaminants. Based on comparison of models with and without covariates for the inclusion/exclusion of contaminants, there was no evidence that this affected sensitivity and specificity (Appendix 5).

2.2.2.3.1.1.2 SeptiFast test compared with blood culture plus MALDI-TOF MS

Only one study (Tafelski *et al*., 2015),[114] which compared the SeptiFast test with MALDI-TOF MS, provided data on diagnostic test accuracy. This study reported a sensitivity and specificity of 0.58 (95% CI: 0.30, 0.86) and 0.74 (95% CI: 0.64, 0.85) respectively.

2.2.2.3.1.2. SepsiTest

2.2.2.3.1.2.1 SepsiTest compared with blood culture

The pooled sensitivity and specificity of SepsiTest compared with blood culture was 0.48 (95% CrI: 0.21, 0.74) and 0.86 (95% CrI: 0.78, 0.92), respectively (Figure 6). The 95% prediction intervals of 0.07, 0.90 (sensitivity) and 0.66, 0.95 (specificity) suggest considerable uncertainty in predicting the sensitivity and specificity of a new study. The between-study standard deviations for logit sensitivity

and specificity were estimated to be 0.90 (95% CrI: 0.50, 1.92) and 0.45 (95% CrI: 0.27, 0.90), with correlation -0.03 (95% CrI: -0.73, 0.68). Figure 7 presents the joint distribution for sensitivity and specificity and highlights the extent of the heterogeneity between studies (as indicated by the 95% prediction interval. The proportion of discordant results with blood culture varied across studies from 14% to 26%, with median 22%. Due to insufficient information provided in the included studies, planned subgroup analyses were not conducted and there were insufficient studies to conduct meaningful analyses.

Figure 6: Sensitivity and specificity of SepsiTest compared with blood culture (

Figure 7: Summary receiver operating curve plot of SepsiTest compared with blood culture [redacted]

2.2.2.3.1.2.2 SepsiTest compared with blood culture plus MALDI-TOF MS

Only one study (Loonen *et al*., 2014),[116] which compared the SepsiTest assay with MALDI-TOF MS provided data on diagnostic test accuracy. This study reported a sensitivity and specificity of 0.11 (95% CI: 0.00, 0.23) and 0.96 (95% CI: 0.92, 1.00) respectively.

2.2.2.3.1.3 IRIDICA assay compared with blood culture

The pooled sensitivity and specificity of IRIDICA compared with blood culture was 0.81 (95% CrI: 0.69, 0.90) and 0.84 (95% CrI: 0.71, 0.92), respectively (Figure 8). The 95% prediction intervals of 0.55, 0.94 (sensitivity) and 0.50, 0.96 (specificity) suggest considerable uncertainty in predicting the sensitivity and specificity of a new study. The between-study standard deviations for logit sensitivity and specificity were estimated to be were 0.46 (95% CrI: 0.28, 0.93) and 0.65 (95% CrI: 0.39, 1.27), with 0.06 (95% CrI: -0.71, 0.75). Figure 9 presents the joint distribution for sensitivity and specificity and highlights the extent of the heterogeneity between studies (as indicated by the 95% prediction interval). The proportion of discordant results with blood culture (i.e. cases of disagreement between the reference standard and the index test) varied across studies from 7% to 30%, with median 18%. Due to insufficient information provided in the included studies, planned subgroup analyses were not conducted and there were insufficient studies to conduct meaningful analyses.

Figure 8: Sensitivity and specificity of IRIDICA compared with blood culture [redacted]

Figure 9: Summary receiver operating curve plot of IRIDICA compared with blood culture [redacted]

2.2.2.3.2 Other intermediate measures and clinical outcomes

A total of 41 studies provided data on one or more intermediate and/or clinical outcome measure: 37 SeptiFast studies (Raglio *et al.* 2006;[60] Bingold *et al.* 2007;[61] Klemm *et al.* 2007;[62] Louie *et al.* 2008;[46] Mancini *et al.* 2008;[45] Dierkes *et al.* 2009;[66] Lehmann *et al.* 2009;[68] Paolucci *et al*. 2009;[71] Palomares *et al.* 2009;[70] Westh *et al*., 2009;[74] Bloos *et al*. 2010;[76] Lehmann *et al*. 2010;[78] Maubon *et al.* 2010;[79] Reguerio *et al*. 2010;[80] Tsalik *et al.* 2010;[82] Wallet *et al.* 2010;[83] Hettwer *et al.* 2011;[86] Josefson *et al.* 2011;[87] Vrioni *et al.* 2011;[90] Alvarez *et al.* 2012;[91] Grif *et al*. 2012;[92] Lodes *et al*. 2012;[94] Mauro *et al.* 2012;[95] Pasquilani *et al*. 2012;[96] Tschiedel *et al.* 2012;[98] Herne *et al.* 2013;[99] Paolucci *et al.*, 2013;[101] Rodrigues *et al.* 2013;[102] Avolio *et al.* 2014;[103] Mancini *et al.* 2014;[105] Markota *et al*. 2014;[106] Ozkaya-Parlakay *et al*. 2014;[107] Schaub *et al.* 2014;[108] Sitnik *et al.* 2014;[109] Idelevich *et al.*, 2015;[112] Tafelski *et al*. 2015;[114] Warhurst *et al*. 2015[113]), one SepsiTest study (Loonen *et al*, 2014[116]), two IRIDICA studies (Metzgar *et al*,. unpublished;[119] Vincent *et al*. in press[121]), and one study evaluating both SeptiFast and SepsiTest (Schreiber *et al*. 2013[118]). A brief summary of the studies reporting data on each of the intermediate and clinical outcomes measure is presented in Table 7.

Across the studies reporting intermediate and/or clinical outcomes, the majority of studies reported data for the whole patient cohort, as opposed to comparative data for the index and reference test. Furthermore, for some outcomes, e.g., mortality, it was often unclear at what point the outcome was assessed. These limitations in reporting prohibited any statistical analysis to pool any intermediate and/or clinical outcome across included studies. None of the included studies provided data on re-admission rates, adverse events associated with broad spectrum antimicrobial use, morbidity, changes in disease severity over time, rates of superinfection, rates of resistant infection or health related quality of life.

Table 7: Summary of intermediate and clinical outcomes reported across studies

Author (year)	Time to pathogen identification - Index Test	Time to treatment	Test failure rates	Mortality	Duration of ICU and/or hospital stay	Duration of antibiotic therapy	Reported changes in antimicrobial treatment plan
SINGLE INDEX TEST STUDIES - SEPTIFAST							
Raglio *et al.* (2006)[60]	✓						
Bingold *et al.* (2007)[61]	✓						
Klemm *et al.* (2007)[62]	✓						
Louie *et al.* (2008)[46]	✓						
Mancini *et al.* (2008)[45]	✓						
Dierkes *et al.* (2009)[66]	✓		✓	✓	✓		✓
Lehmann *et al.* (2009)[68]				✓	✓		✓
Palomares *et al.* (2009)[70]	✓						
Paolucci *et al.* (2009)[71]	✓						
Westh *et al.* (2009)[74]			✓				
Bloos *et al.* (2010)[76]				✓	✓		
Lehmann *et al.* (2010)[78]					✓		
Maubon *et al.* (2010)[79]	✓						✓
Reguerio *et al.* (2010)[80]				✓			
Tsalik *et al.* (2010)[82]	✓			✓	✓		
Wallet *et al.* (2010)[83]	✓						✓
Hettwer *et al.* (2011)[86]			✓				
Josefson *et al.* (2011)[87]	✓			✓			
Vrioni *et al.* (2011)[90]	✓						✓
Alvarez *et al.* (2012)[91]				✓	✓		
Grif *et al.* (2012)[92]				✓			✓
Lodes *et al.* (2012)[94]				✓			✓
Mauro *et al.* (2012)[95]	✓						
Pasquilani *et al.* (2012)[96]				✓			
Tschiedel *et al.* (2012)[98]	✓						✓
Herne *et al.* (2013)[99]	✓						✓

Author (year)	Time to pathogen identification - Index Test	Time to treatment	Test failure rates	Mortality	Duration of ICU and/or hospital stay	Duration of antibiotic therapy	Reported changes in antimicrobial treatment plan
Paolucci *et al.* (2013)[101]			✓				
Rodrigues *et al.* (2013)[102]		✓		✓	✓		✓
Avolio *et al.* (2014)[103]	✓						
Mancini *et al.* (2014)[105]				✓	✓		✓
Markota *et al*. (2014)[106]				✓	✓		✓
Ozkaya-Parlakay *et al*. (2014)[107]				✓	✓		
Schaub *et al.* (2014)[108]	✓		✓		✓		
Sitnik *et al.* (2014)[109]	✓						
Idelevich *et al.* (2015)[112]	✓	✓		✓	✓		✓
Tafelski *et al*. (2015)[114]	✓	✓	✓	✓	✓	✓	✓
Warhurst *et al*. (2015)[113]			✓	✓	✓		
SINGLE INDEX TEST STUDIES - SEPSITEST							
Loonen *et al*. (2014)[116]				✓			
SINGLE INDEX TEST STUDIES - IRIDICA							
Metzgar *et al.* (unpublished)[119] Vincent *et al*. (in press)[121]							
TWO INDEX TEST STUDIES – SEPTIFAST AND SEPSITEST							
Schreiber *et al*. (2013)[118]				✓			

2.2.2.3.2.1 Time to result (pathogen identification)

A summary of the studies reporting the times to pathogen identification of the index and reference test are presented in Table 8. Twenty-one SeptiFast studies reported data on the time to availability of results/pathogen identification (Raglio *et al.* 2006;[60] Bingold *et al.* 2007;[61] Klemm *et al.* 2007;[62] Louie *et al.* 2008;[46] Mancini *et al.* 2008;[45] Dierkes *et al.* 2009;[66] Palomares *et al.* (2009);[70] Paolucci *et al.* 2009;[71] Maubon *et al.* 2010;[79] Tsalik *et al.* 2010;[82] Wallet *et al.* 2010;[83] Josefson *et al.* 2011;[87] Vrioni *et al.* 2011;[90] Mauro *et al.* 2012;[95] Tschiedel *et al.* 2012;[98] Herne *et al.* 2013;[99] Avolio *et al.,* 2014[103] Schaub *et al.* 2014;[108] Sitnik *et al.* 2014;[109] Idelevich *et al.,* 2015;[112] Tafelski *et al.* 2015[114]). However, for the majority of these studies it was unclear if the value was the mean or median and variance estimates or ranges were not reported. Across these studies, the reported time to pathogen identification with SeptiFast ranged from four hours (Palomares *et al.*, 2009[70]) to a median of 26.25 hours (range 6.75 to 79 [for samples collected at beginning of weekend]) (Dierkes *et al.*, 2009[66]). In contrast, the time to pathogen identification using blood cultures (with or without MALDI-TOF MS) ranged from 24 hours (minimum) to a median of 80 hours. Time to pathogen identification was not reported by any of the studies evaluating SepsiTest or IRIDICA.

Table 8: Time to test results for index and reference test

Author (year)	Time to pathogen identification - Index Test	Time to pathogen identification - Reference Test
SEPTIFAST STUDIES		
Raglio *et al.* (2006)[60]	16 to 30 hours	5 to 7 days
Bingold *et al.* (2007)[61]	6 hours	24 to 48 hours
Klemm *et al.* (2007)[62]	6.5 hours (minimum)	2 days
Louie *et al.* (2008)[46]	6.54 hours (mean)	65 hours (median) (range 24 to 214)
Mancini *et al.* (2008)[45]	NR	Detection with blood culture (mean, range) 10 hours for E.*coli* to 22.2 hours for Coagulase-negative staphylococci Definitive identification (mean range) 44.2 hours for Coagulase-negative staphylococci to 56.6 hours for E. *faecalis*
Dierkes *et al.* (2009)[66]	18 hours (median): twice daily analysis (range 6.75 to 74 hours for samples collected at beginning of weekend) 26.25 hours (median): once daily analysis (range 6.75 to 79 hours for samples collected at beginning of weekend)	NR
Palomares *et al.* (2009)[70]	4 hours	6.5 hours
Paolucci *et al.* (2009)[71]	Information on antimicrobial susceptibility or micro-organism viability ~8 h	48 to 72 hours
Maubon *et al.* (2010)[79]	6.5 hours	NR
Tsalik *et al.* (2010)[82]	6.5 hours (approx.)	NR
Wallet *et al.* (2010)[83]	7 to 15 hours	24 to 72 hrs
Josefson *et al.* (2011)[87]	6 hours	NR
Vrioni *et al.* (2011)[90]	7 to 15 hours	24 to 72 hours
Mauro *et al.* (2012)[95]	6 hours (approx.)	NR
Tschiedel *et al.* (2012)[98]	17 hours (range 6 to 17)	48 hours (range 48 to 120, median 120)
Herne *et al.* (2013)[99]	NR (range 5 to 22 hours)	NR
Avolio *et al.* (2014)[103]	16.6 hours (mean [95% CI: 14.9 to 18.2) or 15 hours (median [range 13 to 17) (excludes SeptiFast and blood culture negative results) 13-17	84.2 hours (mean [95% CI: 82 to 86.4) or 80 hours (median [range 79 to 84) (excludes SeptiFast and blood culture negative results)
Schaub *et al.* (2014)[108]	6 hours	16 hours (median [range 6 to 44])
Sitnik *et al.* (2014)[109]	<8 hours (mean)	3.5 days (mean) for blood culture positive results 5 days for blood culture negative results

Author (year)	Time to pathogen identification - Index Test	Time to pathogen identification - Reference Test
Idelevich *et al.* (2015)[112]	20.3 hours (Mean)	58.3 hours (Mean)
Tafelski *et al*. (2015)[114]	15.9 ±SD 5.9 hours (Mean) (95% CI: assuming n=41, 14.1 to 17.7*)	38.1 ±SD 11.6 hours (Mean) (95% CI: assuming n=37, 34.4 to 41.8*)

* Estimated by authors

2.2.2.3.2.2 Time to treatment

Time to treatment was reported by three SeptiFast studies, one of which was the RCT by Tafelski *et al.*, (2015)[114] comparing SeptiFast with blood culture and MALDI-TOF MS and two of which were the RCTs by Rodrigues *et al.,* (2013)[102] and Idelevich *et al.* (2015)[112] comparing SeptiFast with blood culture. Tafelski *et al.,* (2015)[114] reported the mean (SD) time from initially drawing blood to adaptation of antimicrobial treatment was 18.8 (5.6) hours in the intervention (SeptiFast) group compared with 38.3 (14.5) hours in the control group (p-value for difference not reported). The number of patients with therapy modification based on a positive diagnostic test was 4/41 (9.8%) in the SeptiFast group and 5/37 (13.5%) in the blood culture group. Rodrigues *et al.,* (2013)[102] reported a mean time to change in therapy of 580 minutes (9.7 hours) with SeptiFast compared with 3,007 minutes (50.1 hours) (between-group difference p=0.004) for blood culture. The number of patients in whom an adjustment of treatment was performed was 6/17 (3.5%) in the SeptiFast group and 7/29 (24%) in the blood culture group.

2.2.2.3.2.3 Test failure rates (internal control, reagents, other)

Seven SeptiFast studies (Dierkes *et al.*, 2009;[66] Westh *et al*., 2009;[74] Hettwer *et al.*, 2011;[86] Paolucci *et al,*. 2013;[101] Schaub *et al.* 2014;[108] Tafelski *et al*. 2015;[114] Warhurst *et al*. 2015[113]) reported information relating to failure rates. In the SeptiFast studies, test failure rates ranged from 1.5% (Schaub *et al,*. 2014[108]) to 24.2% (Hettwer *et al.*, 2011[86]). A summary of the failure rates reported by the SeptiFast studies is presented in Table 9.

[redacted]

Table 9: Test failure rates (internal control, reagents, other)

Author (year)	Reported test failure rate details
SEPTIFAST STUDIES	
Dierkes *et al.* (2009)[66]	One failure was attributed to technical problems during the analysis, however no further details were provided
Westh *et al.* (2009)[74]	70/558 (12.5% of episodes)
Hettwer *et al.* (2011)[86]	38/157 (24.2%)
Paolucci *et al.* (2013)[101]	100/437 (22.9% of samples corresponding to 75 febrile episodes)
Schaub *et al.* (2014)[108]	3/205 (1.5% of sample had technical failure where the internal control was not detected)
Tafelski *et al.* (2015)[114]	4/37 (10.8%) '
Warhurst *et al.* (2015)[113]	69/1006 (6.9% of episodes) (SeptiFast assay failure: Reagent Control (n = 6), Internal Control (n = 56)], Other reasons (n=7))

2.2.2.3.2.4 Duration of ICU and/or hospital stay

Thirteen of the included studies, all of which evaluated SeptiFast compared with blood culture, reported details of ICU and/or hospital stay. Alvarez *et al.,* (2012)[91] reported a significant between-group difference in both ICU and hospital length of stay ($p<0.05$). In contrast Idelevich *et al.*, (2014)[112] reported no statistically significant between-group difference in either ICU or hospital length of stay ($p= 0.815$ and 0.235 respectively). Mancini *et al.*, (2014)[105] also reported no observed between-group differences in the length of stay. The RCT by Rodrigues *et al.*, (2013)[102] also reported no statistically significant between-group difference in hospital stay ($p=0.632$) based on propensity matching. Across the other studies reporting this outcome, data were often reported as characteristics of the included participants and it was often unclear if the length of stay was up to, including, and/or after blood sampling. Details of these studies and the length of stay are reported in Table 10.

Table 10: Details of studies (all SeptiFast) reporting duration of ICU and/or hospital stay

Study author (year)	Duration of ICU and/or hospital stay
SEPTIFAST STUDIES	
Dierkes *et al*. (2009)[66]	Hospital stay: 35 days (median)
Bloos *et al*. (2010)[76]	ICU stay: 13 days (median) Hospital stay: 34 days(median)
Lehmann *et al*. (2010)[78]	ICU stay true negatives: 17 days (range 1 to 89) ICU stay true positives: 36 days (range 8 to 87) Hospital stay true negatives: 23 days (range 1 to 93) Hospital stay true negatives of 38 days (range 8 to 90)
Tsalik *et al*. (2010)[82]	Hospital stay: 6.3 days (mean)
Alvarez *et al.* (2012)[91]	ICU stay SeptiFast: 22.9 ±29.9 days (mean) ICU stay blood culture: 31.0 ±19.4 days (mean) Hospital stay SeptiFast: 18.3 ±21.4 days (mean) Hospital stay blood culture: 21.3 ±23.4 days (mean) Between-group difference ICU and Hospital p<0.05
Rodrigues *et al.* (2013)[102]	Hospital stay SeptiFast: 32 days (mean) Hospital stay blood culture: 31 days (mean) Between-group difference p=0.632
Mancini *et al.* (2014)[105]	Hospital stay: no between-group differences were observed (no data reported)
Markota *et al*. (2014)[106]	Hospital stay: 27 ±28.9 days (mean)
Ozkaya-Parlakay *et al*. (2014)[107]	ICU stay: 15.3 ±23.8 days (mean)
Schaub *et al*. (2014)[108]	Hospital stay: median 11 days
Idelevich et al. (2015)[112]	ICU stay SeptiFast: 0.8 ±4.0 (mean) ICU stay blood culture: 0.9 ±3.4 (mean) Hospital stay SeptiFast: 40.4 ±25.3 (mean) Hospital stay blood culture: 42.9 ±22.0 (mean) Between-group difference Hospital p =0.235; ICU p = 0.815
Tafelski *et al*. (2015)[114]	ICU stay SeptiFast: 34 days (range 13 to 65) ICU stay blood culture: 32 days (range 16 to 57) Hospital stay SeptiFast: 53 days (range 33 to 79). Hospital stay blood culture: 37 days (range 20 to 76)
Warhurst *et al*. (2015)[113]	ICU stay: 16 days (IQR 9 to30)

ICU, intensive care unit; IQR, interquartile range

2.2.2.3.2.5 Duration of broad and narrow spectrum antimicrobial therapy

Only one study, which was the SeptiFast RCT by Tafelski *et al*., (2015)[114] reported information on the duration of antimicrobial therapy which was 18.8 hours (±5.6 SD) in the SeptiFast group and 38.3 hours (±14.5 SD) in the blood culture group. This outcome was not reported by any of the other included studies.

2.2.2.3.2.6 Change in antimicrobial treatment plan

Details of change in antimicrobial treatment plan were reported by fourteen SeptiFast studies (Dierkes *et al.,* 2009;[66] Lehmann *et al.,* 2009;[68] Maubon *et al.,* 2010;[79] Wallet *et al.,* 2010;[83] Vrioni *et al.,* 2011;[90] Grif *et al.,* 2012;[92] Lodes *et al.,* 2012;[94] Tschiedel *et al.,* 2012;[98] Herne *et al.,* 2013;[99]

Rodrigues *et al.,* 2013;[102] Mancini *et al.,* 2014;[105] Markota *et al.,* 2014;[106] Idelevich et al., 2015;[112] Tafelski *et al.,* 2015[114]) [redacted] Details of these studies and the reported changes are presented in Table 11.

Nine of the SeptiFast studies reported on changes in antimicrobial therapy based on the SeptiFast results (Dierkes *et al*., 2009;[66] Markota *et al*., 2014 ;[106] Wallet *et al.,* 2010 ;[83] Vrioni *et al.,* 2011 ;[90] Grif *et al.,* 2012 ;[92] Lodes *et al.,* 2012 ;[94] Tschiedel *et al.,* 2012 ;[98] Herne *et al.,* 2013 ;[99] Maubon *et al.,* 2010 [79]). These studies did not report on changes based on blood culture results. One SeptiFast study reported on changes based on the blood results only (Lehmann *et al.,* 2009[68]). The SeptiFast RCT by Rodrigues *et al.* (2013)[102] reported that 6/17 (35%) patients in the SeptiFast group and 7/29 (21%) patients in the blood culture group, had an adjustment of antimicrobial therapy. The respective numbers for the RCT by Idelevich et al. (2015)[112] were 7/74 (9.5 %) patients in the SeptiFast group and 8/76 (10.5%) patients in the blood culture group. The RCT by Tafelski *et al*. (2015)[114] reported that 4/41 (9.8%) patients in the SeptiFast and blood culture with MALDI-TOF MS group and 5/37 (13.5%) patients in the blood culture with MALDI-TOF MS had an adjustment of antimicrobial therapy. A p-value for the between-group difference was not reported by any of these RCTs.

Table 11: Details of studies reporting changes in antimicrobial treatment plan

Author (year)	Reported changes in antimicrobial treatment plan
SEPTIFAST STUDIES	
Dierkes *et al*. (2009)[66]	SeptiFast: from pathogens identified by SeptiFast only, 5 (7.7%) patients had an adjustment of antimicrobial therapy
Lehmann *et al.* (2009)[68]	Blood culture: in 49/467 (9.5%) of episodes, antimicrobial treatment was changed
Maubon *et al*. (2010)[79]	SeptiFast: results would have significantly improved treatment in 11 (10%) of patients and prompted immediate antimicrobial therapy not given initially in 3 patients
Wallet *et al*. (2010)[83]	SeptiFast: on the basis of results, 8/72 (11.1%) patients had an adjustment of antimicrobial therapy
Vrioni *et al*. (2011)[90]	SeptiFast: on the basis of results, 5/33 (15.2%) patients had an adjustment of antimicrobial therapy
Grif *et al*. (2012)[92]	SeptiFast and concordant results from blood culture: 3/33 (9.1%) patients had an adjustment of antimicrobial therapy SeptiFast and concordant results from samples from body sites: 5/33 (15.2%) patients had an adjustment of antimicrobial therapy
Lodes *et al*. (2012)[94]	SeptiFast: on the basis of results, 25/148 (16.9%) of samples had an adjustment of antimicrobial therapy
Tschiedel *et al*. (2012)[98]	Patients with positive SeptiFast : 35/75 (46%) had an adjustment of antimicrobial therapy Patients with negative SeptiFast : 5/75 (6%) had an adjustment of antimicrobial therapy
Herne *et al*. (2013)[99]	SeptiFast: on the basis of results, 21/54 (39%) positive cases had an adjustment of antimicrobial therapy
Rodrigues *et al*. (2013)[102]	SeptiFast: on the basis of results, 6/17 (35%) patients had an adjustment of antimicrobial therapy Blood culture: on the basis of results, 7/29 (21%) patients had an adjustment of antimicrobial therapy Between-group difference not reported
Mancini *et al.* (2014)[105]	Reports no between-group differences were observed in changes in management (propensity matching).
Markota *et al*. (2014)[106]	SeptiFast: on the basis of results, 4 (6.3%) samples had an adjustment of antimicrobial therapy
Idelevich et al. (2015)[112]	SeptiFast: on the basis of results, 7/74 (9.5 %) patients had an adjustment of antimicrobial therapy Blood culture: on the basis of results, 8/76 (10.5%) patients had an adjustment of antimicrobial therapy Between-group difference not reported
Tafelski *et al*. (2015)[114]	SeptiFast and blood culture with MALDI-TOF MS: on the basis of results, 4/41 (9.8%) patients had an adjustment of antimicrobial therapy Blood culture with MALDI-TOF MS: on the basis of results, 5/37 (13.5%) patients had an adjustment of antimicrobial therapy Between-group difference not reported
IRIDICA STUDIES	

Author (year)	Reported changes in antimicrobial treatment plan
Vincent *et al*. (in press)[121]	[redacted]

2.2.2.3.2.7 Mortality

Seventeen of the SeptiFast studies (Dierkes *et al.,* 2009;[66] Lehmann *et al.,* 2009;[68] Bloos *et al.,* 2010;[76] Reguerio *et al.,* 2010;[80] Tsalik *et al.,* 2010;[82] Josefson *et al.,* 2011;[87] Alvarez *et al.,* 2012;[91] Grif *et al.,* 2012;[92] Lodes *et al.,* 2012;[94] Pasquilani *et al.,* 2012;[96] Rodrigues *et al.,* 2013;[102] Mancini *et al.,* 2014;[105] Markota *et al.,* 2014;[106] Ozkaya-Parlakay *et al.,* 2014;[107] Idelevich et al., 2015;[112] Tafelski *et al.,* 2015;[114] Warhurst *et al.,* 2015[113]), one SepsiTest study (Loonen *et al*., 2014[116]), [redacted] and one study evaluating both SeptiFast and SepsiTest (Schreiber *et al*. 2013[118]) reported data on mortality. A summary of the mortality rates are presented in Table 12.

Across the studies comparing SeptiFast with blood culture, one study reported that 16 (61%) died within 24 hours after blood sampling (Grif *et al*., 2012[92]). In-hospital mortality was reported by three studies. Dierkes *et al*., (2009)[66] reported 33% in-hospital mortality for the entire cohort, Pasquilani *et al*., (2012)[96] reported 48/391 (12%) hospital deaths in all patients, Markota *et al*., (2014)[106] reported in-hospital mortality of 52.6%. Josefson *et al*., (2011)[87] reported 30-day mortality of 45/1093 (4%) and Warhurst *et al*., (2015)[113] reported a 28-day mortality of 14% (n=792). One SeptiFast study reported both 28-day and 6-month mortality, but that the between-group differences at both time points were not statistically significant (Alvarez *et al.,* (2012)[91]). Lehmann *et al.,* (2009)[68] reported that the 30-day mortality associated with all SeptiFast episodes was 26.7% and 30-day mortality across both blood culture and SeptiFast episodes was 33.8%. The SeptiFast RCT by Tafelski *et al*., (2015)[114] reported ICU mortality of 7/41 (17%) in the SeptiFast group and 8/37 (22%) in the blood culture group. The SeptiFast RCT by Idelevich *et al*., (2014)[112] reported that 5 (6.6%) in the blood culture group and 3 (4.1%) in the SeptiFast group died, but did not report when this occurred. The SeptiFast RCT by Rodrigues *et al.,* (2013)[102] reported that the between-group difference in 28-day mortality was not statistically significant. The two index test study evaluating both SeptiFast and SepsiTest by Schreiber *et al*., (2013)[118] reported an ICU mortality of 8/50 (16%) and 28-day mortality of 12/50 (24%) across all patients. Across the remaining studies reporting this outcome, the location and time of mortality was unclear.

Table 12: Details of studies reporting data on mortality

Author (year)	Reported mortality details
SINGLE INDEX TEST STUDIES -SEPTIFAST	
Dierkes *et al*. (2009)[66]	In-hospital mortality: 33%
Lehmann *et al.* (2009)[68]	30-day mortality: 33.8% of 467 episodes
Bloos *et al*. (2010)[76]	29.9% - location / time period NR
Reguerio *et al*. (2010)[80]	37.5% - location / time period NR *
Tsalik *et al*. (2010)[82]	2.6% - location / time period NR
Josefson *et al*. (2011)[87]	30-day mortality: 4%
Alvarez *et al.* (2012)[91]	28-day mortality SeptiFast: 29% 28-day mortality blood culture: 24 6-month mortality SeptiFast: 41.6% 6-month mortality blood culture: 37% Between-group difference p=NS
Grif *et al*. (2012)[92]	24-hour mortality: 61%
Lodes *et al*. (2012)[94]	43.2% - location / time period NR
Pasquilani *et al*. (2012)[96]	In-hospital: 12%
Rodrigues *et al.* (2013)[102]	28-day mortality SeptiFast: 53% 28-day mortality blood culture: 59% Between-group difference p=0.765
Mancini *et al*. (2014)[105]	The mortality difference in the original propensity score matching was not significant 8.24% (prospective cohort) *vs*. 13.48% (retrospective cohort) p = 0.39. However, in a more stringently matched group SeptiFast was reported to have better mortality rates (3.13% [n=2 deaths in prospective cohort] compared with 14.71% [n=10 deaths] in retrospective cohort] p-value =0.04).
Markota *et al*. (2014)[106]	In-hospital mortality 52.6%
Ozkaya-Parlakay *et al*. (2014)[107]	25.3% - location / time period NR
Idelevich et al. (2015)[112]	SeptiFast: 4.1% - location / time period NR Blood culture: 6.6% - location / time period NR Between-group difference 0.719
Tafelski *et al*. (2015)[114]	ICU-mortality SeptiFast and blood culture with MALDI-TOF MS: 17% ICU-mortality blood culture with MALDI-TOF MS: 22% Between-group difference NR
Warhurst *et al*. (2015)[113]	28-day mortality: 14%
SINGLE INDEX TEST STUDIES -SEPSITEST	
Loonen *et al*. (2014)[116]	3.2% - location / time period NR
SINGLE INDEX TEST STUDIES -IRIDICA	
[redacted]	[redacted]
TWO INDEX TEST STUDIES -SEPTIFAST AND SEPSITEST	
Schreiber *et al*. (2013) [118]	ICU mortality 16% 28-day mortality 24%

* reporting discrepancy in article, text says 32.8% tables says 37.5%

2.2.3 Additional information on MALDI-TOF MS

Although not an intervention, and therefore omitted from the systematic review of clinical effectiveness, information on the diagnostic accuracy and in the potential benefits associated with MALDI-TOF MS was required. Two recent systematic reviews have been published: one focussing

on the time taken to identify microbial organisms from positive blood cultures[124] and one reviewing the performance of Sepsityper kit in conjunction with MALDI-TOF MS.[30]

Dixon *et al*.,[124] identified ten studies which provided evidence that MALDI-TOF MS is associated with faster identification of pathogens, usually 24 hours quicker than blood culture alone. Where data were reported, MALDI-TOF MS was associated with a reduction in hospital costs and length of stay. However, the authors state that 'all the included studies were observational and their findings have a relatively high risk of bias' and that 'MALDI-TOF MS has the potential to reduce length of stay and costs while improving patient outcomes, but more and better evidence, including that on cost-effectiveness, is required.'

Mergenthaler and Kostrzewa summarise data from 21 reports to assess the reliability of the Sepsityper kit in the rapid identification of blood stream infection. It was reported that 'no relevant misidentification on the genus level was reported at a log (score) cut-off of 1.6' whilst time to a result was reduced by several hours or days.

In addition to these reviews, papers known to the authors, submitted by the company or identified in the sifting related to the review of economic evaluations of the interventions were read to provide additional information regarding MALDI-TOF MS. Citation searching was performed to identify further information. It is noted that often MALDI-TOF MS was introduced in conjunction with another change, such as the establishment of an antimicrobial stewardship team, and therefore the exact gain attributable to MALDI-TOF MS was unknown.

Perez *et al.*[125] report the implementation of an evidence-based intervention that integrated MALDI-TOF MS, rapid antimicrobial susceptibility testing, and near–real-time antimicrobial stewardship practices. Comparison of results before and after were made. The mean hospital length of stay for survivors (n = 100) after blood stream infection onset in the pre-intervention group was 9.9 versus 8.1 days in the intervention group (n=101; p-value=.01). Within a multivariate model receiving active antibiotic therapy at 48 hours was associated with a hazard ratio for discharge of 2.90 (95% CI 1.15-7.33; p-value = 0.02) and the intervention was associated with a hazard ratio for discharge of (95% CI 1.01-1.88; p-value = 0.04). Total hospitalisation costs was $45,709 in the pre-intervention cohort vs $26,162 in the intervention cohort.

A further paper[126] reported a pre–post quasi-experimental study which analysed the impact of MALDI-TOF MS with an antimicrobial stewardship team. The intervention (n = 256) decreased time to organism identification (84.0 vs 55.9 hours, p-value < .001), and improved time to effective antibiotic therapy (30.1 vs 20.4 hours, p-value = .021), optimal antibiotic therapy (90.3 vs 47.3 hours,

p-value < .001) and length of ICU stay (14.9 vs 8.3 days, p-value = .014) compared with pre-intervention (n=245). 30-day all-cause mortality was lower in the intervention arm compared with pre-intervention (12.73 vs 20.3%. p-value = .021) as was length of hospitalisation (14.2 vs 11.4 days, p-value = .066).

A study in Texas, United States of America (USA) compared the outcomes of 112 patients with antibiotic-resistant Gram-negative bacteremia, during January 2009 – November 2011 with 157 patients during February 2012 to June 2013 following the introduction of an intervention (MALDI-TOF MS and antimicrobial stewardship).[127] Time to initiation of active treatment was 90 hours pre-intervention and 32 hours post intervention (p<0.001). There were 33 (21%) and 10 (9%) all-cause mortalities observed in the pre-intervention cohort and the intervention cohort respectively. In multivariate logistic regression the intervention was a significant predictor of survival (OR=0.28, 0.12-0.71; p-value =0.008). A significant reduction in average total hospital costs was observed from $78,991 to $52,693.

A quasi-experimental study[128] was conducted evaluating MALDI-TOF MS plus antimicrobial stewardship team review for patients hospitalised with blood cultures positive due coagulase-negative Staphlococcus (n=324). 246 were deemed contamination (117 in the pre-intervention and 129 in the intervention group) whereas 78 patients (46 in the pre-intervention group and 32 in the intervention group) had bacteremia. Patients with bacteremia received optimal therapy more quickly in the intervention group (58.7 versus 34.4 hours, p = 0.032) and had a lower mortality rate (21.7% versus 3.1%, p = 0.023). Patients with contaminated samples had a decreased duration of unnecessary antibiotic therapy (1.31 versus 3.89 days, p = 0.032) and a decreased number of vancomyn trough assays performed (0.88 versus 1.95, p<0.001) but similar rates of mortality, duration of hospitalisation stay, recurrent bloodstream infections and 30-day hospital readmissions.

A paper by Martiny and Debaugnies[129] reports that the use of MALDI-TOF MS resulted in the modification of treatment in 21/157 adults and 1/40 paediatrics.

The authors wish to draw attention to the RAPIDO study,[31] which will report ideal data for assessing the clinical effectiveness of MALDI-TOF MS, typically in conjunction with Sepsityper and blood culture compared with blood culture alone. It is envisaged that the results from this study will make the preceding data in this section largely redundant.

3. ASSESSMENT OF COST EFFECTIVENESS

3.1 Systematic review of existing economic evidence

This section of the report describes a review of the existing published evidence on the economic impact of the SeptiFast, SepsiTest and IRIDICA tests to rapidly detect and identify bacterial and fungal DNA which may be present in the bloodstream in people who are suspected of having sepsis. As previously stated earlier versions of IRIDICA BAC BSI assay were assumed by the authors to provide generalisable data and these have been included in the review, with explicit reference made to the version of IRIDICA.

3.1.1 Methods

A systematic search of the existing published literature evaluating the economic impact of the SeptiFast, SepsiTest and IRIDICA tests to rapidly detect and identify bacterial and fungal DNA which may be present in the bloodstream in people who are suspected of having sepsis was undertaken.

Studies were identified by searching the following electronic databases and research registers:

- MEDLINE(R) In-Process & Other Non-Indexed Citations and MEDLINE(R) (OvidSP) 1948 to May 2015
- EMBASE (OvidSP) 1980 to May 2015
- Cochrane Database of Systematic Reviews (Wiley Online) 1996 to May 2015
- Cochrane Central Register of Controlled Trials (Wiley Online) 1898 to May 2015
- Health Technology Assessment Database (Wiley Online) 1995 to May 2015
- Database of Abstracts of Review of Effects (Wiley Online) 1995 to May 2015
- NHS Economic Evaluation Database (Wiley Online) 1995 to May 2015
- Science Citation Index Expanded (Web of Science) 1899 to May 2015
- Conference Proceedings Index-Science (Web of Science) 1990 to May 2015
- WHO International Clinical Trials Registry Platform (ICTRP) 2007 to May 2015
- Current Controlled Trials (CCT) 2000 to May 2015
- NIH ClinicalTrials.gov 2000 to May 2015
- Manufacturer and User Facility Device (MAUDE) 1991 to May 2015
- MEDION database

Sensitive keyword strategies using free text and, where available, thesaurus terms using Boolean operators and database-specific syntax were developed to search the electronic databases. Synonyms relating to the condition (e.g. sepsis) and the test (e.g. SeptiFast, SepsiTest and IRIDICA) were combined with a search filter aimed at restricting results to economic and cost-related studies (used in the searches of MEDLINE and EMBASE). No language restrictions were used on any database;

however, the searches were restricted by date (see Section 2.1 for further details). In brief, CE approval for the oldest rapid molecular test (i.e. SeptiFast) was obtained in 2006. As a result, no relevant economic evaluations were expected to be published prior to this date. An example of the MEDLINE search strategy is provided in Appendix 6.

b) Other resources

To identify additional published, unpublished and ongoing studies, the reference lists of all relevant studies were checked and a citation search of relevant articles (using the Web of Science Citation Index Expanded and Conference Proceedings Citation Index - Science) was undertaken to identify articles that cite the relevant articles. In addition, systematic keyword searches of the WWW were undertaken using the Google search engine, key experts in the field were contacted and company submissions were screened for published or unpublished data additional to those identified in studies retrieved from the literature search.

All identified citations from the electronic searches and other resources were imported into and managed using the Reference Manager bibliographic software, (version 12.0; Thomson Reuters, Philadelphia, PA).

Studies were selected for inclusion according to pre-determined inclusion and exclusion criteria. A summary of these criteria are provided in Table 13.

Studies were selected for inclusion through a two-stage process.

- **Level 1 screening:** Titles and abstracts were independently examined for inclusion by two reviewers (RR and MS). Any disagreements in the selection process were resolved through discussion.
- **Level 2 screening:** Full manuscripts of selected citations were then retrieved and assessed by one reviewer (RR). A second reviewer (MS) performed an independent quality check to ensure that the inclusion criteria were applied correctly. Any disagreements in the selection process were resolved through discussion.

Table 13: Inclusion and exclusion criteria for the review of economic evaluation

Criteria	Included	Excluded
Countries	All	No restriction
Settings	All	No restriction
Study design	Economic evaluations (model or study-based) comparing one of the intervention listed below vs. an appropriate comparator, including other interventions if applicable	Non-economic evaluation Cost study of one test only (comparison of costs of different reagents or techniques)
Population	Adults and children (of any age) with suspected blood stream infections in secondary care (i.e. departments and wards providing care for acutely unwell patients and/or critical care units) who required blood cultures were included	
Target condition	People with suspected sepsis	People who do not have suspected sepsis
Comparator test	▪ Blood culture with or without MALDI-TOF MS	Other tests done in house
Interventions (index test)	▪ SeptiFast, ▪ SepsiTest and ▪ IRIDICA	Economic evaluations that do not investigate one of the interventions of interest in at least one of the arms
Outcomes	▪ Cost-minimisation, ▪ Cost-effectiveness, ▪ Cost-utility analysis	

No formal quality assessment was conducted. When assessing the methodological quality of the economic literature, a number of checklists are available; however, quality assessment checklists for assessing economic evaluations of diagnostic tests are limited. Similarly, the majority of checklists focus on the quality of reporting rather than the methodological quality of a study. Due to these limitations the relevance of each study to the decision problem is discussed within Sections 3.1.2.2, 3.1.2.3 and 3.2.

3.1.2 Results

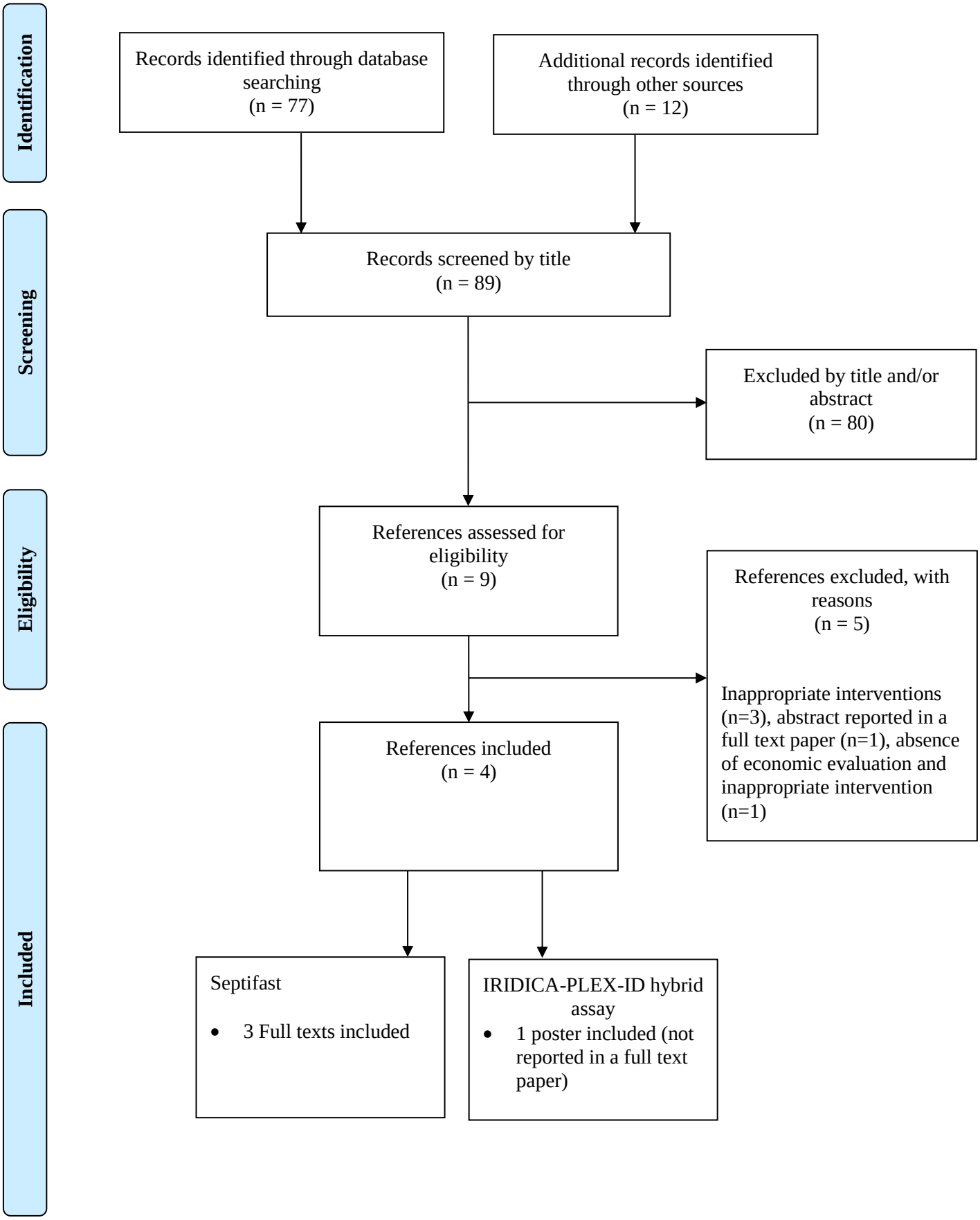
3.1.2.1 Identified studies

A total of 89 citations were retrieved. Of these 77 citations were identified via database searching and and an additional 12 citations were retrieved through other sources. (Figure 10).

Eighty references were excluded at title and abstract stage. Nine references related to eight studies were examined at full-text level and four studies (corresponding to four references) were identified as meeting the inclusion criteria of the systematic review of economic evaluations.[91,105,130,131] These included an economic evaluation of the IRIDICA-PLEX-ID hybrid assay reported in a poster presentation (submitted by the company).[130] It is highlighted that the system evaluated in the poster presentation was not the final IRIDICA BAC BSI assay but was an earlier version that used components of PLEX-ID and is assumed to be equivalent (see Section 1.7.3).

Five papers were excluded after retrieval of the full papers. The rationale being: results published in full elsewhere;[132] other interventions;[133-135] absence of economic evaluation and inappropriate intervention.[136]

Figure 10: Study flow chart (adapted[59]): Economic review



3.1.2.2 Descriptive summary of the study included in the review

A tabulated summary of the key characteristics of the studies included in the economic review, as determined by the authors of this report, is presented in Table 14. It was not possible for the External Assessment Group to check the economic models as only the publications were available in the public domain.

Of the four identified economic studies (corresponding to three full texts[91,105,131] and one poster presentation[130]), three economic evaluations (full text) compared the addition of SeptiFast to blood culture against blood culture alone[91,105,131] and one economic evaluation compared the addition of the IRIDICA-PLEX-ID hybrid assay to blood culture against blood culture alone (poster presentation).[130] No economic evaluations of SepsiTest were identified. None of the four published economic evaluations were conducted in a UK setting. However, the RADICAL study,[121] used for the impact of the IRIDICA-PLEX-ID hybrid assay on treatment modification included two UK (out of the nine) sites.[121]

Two[105,131] out of the three SeptiFast studies were funded by Roche Diagnostics; it was unclear from the third study[91] whether the study was funded by the company. The IRIDICA-PLEX-ID hybrid cost-minimisation study was funded by Abbott Diagnostics.

The target population, condition and setting varied between the four identified economic studies. Mancini *et al.,* (2014)[105] included haematological patients with signs of systemic inflammatory response syndrome with suspected sepsis (SIRS-SS). Alvarez *et al.,* (2012)[91] included patients diagnosed with severe sepsis and septic shock. Lehmann *et al.,* (2010)[131] included all post-surgical and ICU patients with a sepsis episode (predominantly hospital acquired infection) whilst Bilkovski *et al.*, (2014)[130] included critically ill patients with suspected blood stream infection.

Three studies were cost-minimisations. Two were conducted within-studies: a non-matched retrospective study evaluating SeptiFast[91] and a propensity score matched study evaluating SeptiFast against blood culture.[105] The Alvarez study[91] justified the use of cost-minimisation given the absence of mortality data associated with the use of SeptiFast. The third cost-minimisation was undertaken using a decision tree model[130] and evaluated the IRIDICA-PLEX-ID hybrid assay by combining evidence from the RADICAL study[121] on the impact of the test in term of treatment decision and evidence from MALDI-TOF MS studies[125,126] on the impact of rapid identification on the reduction in hospital and ICU length of stay. The main assumptions within the model were that (a) all patients start on empiric antimicrobial therapy, and (b) only patients that are tested positive using the IRIDICA-PLEX-ID hybrid assay were assumed to experience a reduction in length of stay.

Table 14: Key characteristics of economic evaluations included in the review

Parameters	Mancini *et al.*, (2014)[105]	Alvarez *et al.*, (2012)[91]	Lehmann *et al.*, (2010)[131]	Bilkovski *et al.*, (2014)[130]
Country	Italy	Spain	Unclear	Unclear
Study type	Within-study (observational propensity-score matched study) economic evaluation	Within-study (observational retrospective non-matched study) economic evaluation	Mathematic model (evidence from different sources combined)	Mathematical model (based on the RADICAL[121] study and data from two MALDI-TOF MS studies)
Economic evaluation	Cost-minimisation	Cost-minimisation	Cost-effectiveness/cost-utility analysis • Cost per incremental survivors • Cost per QALY gained	Cost-minimisation
Rationale for the approach used	Not provided	No difference in observed mortality	NA	Not provided
Intervention	SeptiFast	SeptiFast	SeptiFast	IRIDICA-PLEX-ID hybrid
Comparator	Blood culture (BC)	BC	BC	BC
Funder of the study	Roche Diagnostics	Unclear	Roche Diagnostics	Abbott Diagnostics
Target population and condition	Haematological patients with signs of systemic	Patients diagnosed with severe sepsis and septic	Patients with a sepsis episode. Predominantly	Critically ill patients with suspected BSI

Parameters	Mancini *et al.*, (2014)[105]	Alvarez *et al.*, (2012)[91]	Lehmann *et al.*, (2010)[131]	Bilkovski *et al.*, (2014)[130]
	inflammatory response syndrome with suspected sepsis	shock	hospital acquired infection	
Settings	Haematology and bone marrow transplant units	ICU	Post-surgical and ICU patients	Majority ICU
Age	≈50±14 years	≈65±14 years	>60 years	60.4 ± 18.8 years
Source used for the impact of the test on treatment modification	NA – within study economic evaluation	NA – within study economic evaluation	Evidence collected prospectively from five hospitals (two German, one Italian, one Spanish and one US hospital)[68]	RADICAL study[121]
Source used for the impact of the test on clinical outcomes (mortality, length of stay)	NA – within study economic evaluation	NA – within study economic evaluation	Pooled data on the impact of inadequate treatment on outcomes from two previously published studies[137,138] conducted in the USA	Two studies of MALDI-TOF MS in addition to an antistewardship program[125,126]
Study perspective	Healthcare perspective	Healthcare perspective	Healthcare perspective	Healthcare perspective
Discounting	NA	NA	Not stated	NA
Time horizon	NA	NA	Lifetime	NA
Cost categories included	• diagnostic and laboratory	• Antibiotic treatment	• SeptiFast test	• ICU stay

Parameters	Mancini *et al.*, (2014)[105]	Alvarez *et al.*, (2012)[91]	Lehmann *et al.*, (2010)[131]	Bilkovski *et al.*, (2014)[130]
in the economic evaluation	assays (including SeptiFast test) • Instrumental diagnostic procedures • Administered therapeutic agents (empiric and pathogen-targeted therapy) • Non-anti-infectious drugs to manage SIRSS-SS related complications	• ICU stay • Ward stay • SeptiFast test		• Ward stay • IRIDICA-PLEX-ID hybrid
Cost of the intervention	€178.75 per sample (average of 4 samples plus positive and negative controls per each run, including both reagents and personnel costs)	€183 (based on 7 patients) per patients including reagent cost, personnel cost and imputable structural costs	€300	$250
Unit costs for other resource use	Taken directly from hospital – values not reported	• Stay in Ward: £273 • Stay in ICU: 1,058 • Cost of antibiotics taken	NA	• Non-ICU stay per day: $2,122 • ICU stay per day: $3,500

Parameters	Mancini *et al.*, (2014)[105]	Alvarez *et al.*, (2012)[91]	Lehmann *et al.*, (2010)[131]	Bilkovski *et al.*, (2014)[130]
		directly from hospital – values not reported		
Measurement of benefits	NA	NA	Mortality Morbidity (associated with sepsis) - QALYs	NA
Utility values	NA	NA	Sepsis: 0.68	NA
Results	Total costs (test vs. BC) per patient: €1,579.80 (median: €1,075.47) vs. €2,010.53 (median €1,105.18); p=0.05 (Saving of €430.73)	Total costs (test vs. BC) per patients: €32,228 vs. €42,198; p=0.05 (Saving of €9,970) 96.3% probability of cost-savings	€11,44 (€9,321 – €14,977) per incremental survivor €3,107 (€2,523 – €4,055) per QALY gained	Total costs (test vs. BC): $19,375,716 vs. $20,499,088 per 422 tests
Breakdown of clinical results	NA	• ICU length of stay (31.0±19.4 vs. 22.9±29.9) • Hospital length of stay (21.3±23.4 vs.	• 80.5 days (CI: 48 – 113 days) potential earlier treatment • Absolute reduction in mortality of 2.6%	• 4.2 days saved in hospital stay in patients with a positive test (1.6 in all patients) • 1.8 days saved in

Parameters	Mancini *et al.*, (2014)[105]	Alvarez *et al.*, (2012)[91]	Lehmann *et al.*, (2010)[131]	Bilkovski *et al.*, (2014)[130]
		18.3±21.4) • ICU length of stay (survivors): 24.1±21.9 vs 18.3±11.4 • Number of antibiotics used per patients (5.1±3.1 vs. 4.2±2.2)		ICU stay in patients with a positive test (0.7 in all patients)
Breakdown of cost results	• Classical diagnostic and instrumental procedures assays (test vs. BC): €652.79 vs. €625.66; p =0.68 • Medications costs: €927.01 vs. €1,384; p =0.02	• Antibiotic treatment costs: €2,812 vs. €3,576; p<0.05 • ICU costs: €24,246 vs. €32,798; p<0.05 • Ward costs: €5,988 vs. €5,824; p<0.05	•	• Hospital cost (per 422 tests): $19,185,816 vs. $20,414,688

BC: blood culture; ICU intensive care unit

Only one study was a cost-effectiveness (cost-utility) analysis which estimated the 'cost per incremental survivor' and the cost per QALY gained of introducing SeptiFast.[131] An algebraic model was constructed, which estimated independently the potential cost impacts and clinical outcomes associated with a change in treatment plan due to earlier identification of inadequate treatment through the use of SeptiFast. Only positive SeptiFast results were considered as providing sufficient evidence to allow a treatment change with the authors concluding that 'withdrawal of antimicrobial treatment upon a PCR negative result is not recommended'. Cost savings and increased health associated with quicker adequate treatment were estimated assuming a relationship between a reduction of one day in inadequate treatment and changes in both length of stay and in mortality. This study used evidence collected prospectively from five hospitals to inform the change in treatment decision: two based in Germany, one in Spain, one in Italy and one in the United States of America (USA).[68] In addition, the modelling uses pooled data on the impact on inadequate treatment on outcomes from two previously published studies[137,138] conducted in the USA.

Although not clearly stated by the authors, it is believed by the authors of this report that Mancini *et al.,* (2014)[105] and Alvarez *et al.,* (2012)[91] report results from an Italian and Spanish setting respectively. It is unclear from the Lehmann *et al.,* (2010)[131] and Bilkovski *et al.,* (2014)[130] the country for whom the analysis is conducted as data were based on multicentre studies.

The economic evaluation was conducted in patients in ICU in three studies.[91,130,131] All studies appear to use a hospital (health care payer) perspective; although this was not explicitly stated in two studies.[105,130] Uncertainty was examined in two studies.[91,131] Only one study used QALYs as a measure of benefit.[131]

The cost of performing SeptiFast was variable between studies and ranged from €178.75[105] (£128.70 assuming an exchange rate of €1 to £0.72[139]) to €300[131] (£216). One study evaluated the IRIDICA-PLEX-ID hybrid assay and assumed a cost of $250 per test[124] (£160.67 assuming an exchange rate of $1 to £0.64).[140]

Two studies[91,105] considered antibiotics costs and both reported a reduction in antibiotics costs associated with the use of SeptiFast. One study included the savings in classical diagnostic assays[105] with the use of SeptiFast. Three of the four studies considered the costs associated with ICU/hospital stay and all reported a reduction in hospital/ICU stay with the use of the test.[78,91,130] None of the studies identified considered the impact on costs associated with the potential reduction in antibiotic resistance.

Overall, all three cost-minimisation studies reported a reduction in total costs with the additional cost of SeptiFast and the IRIDICA-PLEX-ID hybrid assay was outweighed by the savings in antibiotics and/or hospital costs[91,105,130] Mancini *et al.,* (2014)[105] reported an overall saving of €430.73 per patient receiving a SeptiFast test (€1,579.80 vs. €2,010.53) considering only savings in diagnostic and instrumental assays and medications (including antibiotics, antimycotics, antiviral agents and other drugs). Alvarez *et al.,* (2012)[91] reported a saving of €9,970 per SeptiFast test with the majority of savings achieved based on a reduction in ICU length of stay. Bilkovski *et al.,* (2014) reported a total saving of $1,123,372 per 422 patients tested (equating to a saving of £2,662 per patient tested) with the IRIDICA-PLEX-ID hybrid assay associated with a reduction in hospital or ICU length of stay. Finally, Lehmann *et al.,* (2010)[131] reported that the cost of the SeptiFast test could be recovered if the daily medical costs were above €717 and suggested this was likely to be the case.[131] The authors reported the cost per incremental survivor and cost per QALY gained to be €11,477 (95% CI: €9,321 – €14,977) and €3,107 (95% CI: €2,523 – €4,055) respectively.

3.1.2.3 Critique of the study included in the review

The Lehmann *et al.,* (2010)[131] economic evaluation which evaluated the use of SeptiFast against conventional blood culture appears to be a reasonably well conducted cost effectiveness analysis based on the description provided by the authors. However, this study has a number of limitations which may limit the generalisability to English practice not only because none of the hospitals contributing to the study were located in the UK. The data used in the Lehmann (2010)[131] cost-effectiveness model on the potential impact of the test in terms of treatment modification are relatively out-of-date and were collected prior to recent guidelines on the diagnosis and treatment of sepsis.[16,18-20] As such, it is not known the extent to which the inadequate treatment observed in Lehmann *et al.,*[68] is generalisable to current practice in England. Furthermore, the studies on how earlier adequate treatment translates into reduced morbidity and mortality were dated and cohort-based and, as acknowledged by Lehmann *et al.*[131] could be potentially confounded. Lehmann *et al.,*[131] used a relative risk of non-survival between immediate and delayed adequate antimicrobial treatment of 2.32 but report in their discussion section that a value of 1.8 estimated in a clinical trial of immunomodulating therapy for severe sepsis[141] would have been a better estimate of the relative risk of non-survival associated with inadequate treatment. Using this value led to an increase in the cost per incremental survivor from €11,477 to €14,670 (an alternative cost per QALY gained value was not reported). It should be noted that the relative risk is still relatively dated as it was published in 2003.[141] The relative risk of mortality was pooled from three studies, rather than the more appropriate method of meta-analysing. The same limitations of the relationship between inadequate treatment and mortality also apply to the relationship between inadequate treatment and length of hospitalisation which uses data from the two old studies. It is noted that within the original Lehmann *et al.,*[68] paper data were collected from two separate sites of attendance: ICU or surgical ward; and emergency room

or other. The estimated gainable days of adequate treatment per 100 SeptiFast tests were 36.4 for the ICU or surgical ward group and 10.6 for the remaining attendance method. It is unclear whether the evaluation by site was pre-planned or whether the analysis on ICU patients alone could be viewed as data dredging: ideally a replication of the study within the ICU would provide more conclusive data. The failure rate of the SeptiFast test was also not considered. In the model, patients receiving SeptiFast are assumed to experience a mortality benefit associated with rapid identification. Although this may be plausible, so far, evidence on the impact of SeptiFast on clinical outcomes of mortality and hospital length of stay is inconclusive from five comparative studies.[91,105,112,114,142]

The cost-minimisation conducted by Alvarez *et al.*, (2012) is based on a retrospective non-matched comparison of the costs in a cohort prior to the introduction of SeptiFast and a cohort following the use of SeptiFast.[91] Alvarez *et al.* reported a non-significant increase in mortality in the cohort receiving the intervention at 28 days (29% vs. 24%; non-significant; p value not reported) and at 6 months (41.6% vs. 37%, non-significant; p value not reported). In contrast, the authors reported a significant reduction in ICU length of stay for survivors (31.0±19.4 vs. 22.9±29.9; $p<0.05$), hospital length of stay (21.3±23.4 vs. 18.3±21.4; $p<0.05$) and ICU length of stay (24.1±21.9 vs. 18.3±11.4; $p<0.05$) respectively. The authors calculated the total cost to be €42,198 for the control group and €32,228 for the intervention group corresponding to a saving of €9,970 (with more than 85% of savings attributable to a reduction in ICU length of stay). This study has serious limitations that need further consideration. The results need to be interpreted with caution due to the retrospective nature of this study, the small sample size of patients included in each arm (48 patients in the intervention group and 54 patients in the control group) and the imbalances between patient characteristics as showed in Table 15. It is also unclear whether patients were randomly selected to receive SeptiFast or whether these were selected based on their characteristics. Although the authors reported no differences in terms of patients' ages, gender distribution or risk indices, it is unclear from the paper how these risk indices were calculated. There appear to be large imbalances in the initial diagnoses which could impact on ICU length of stay (main source of savings). It should be noted that no difference in ICU or hospital length of stay were observed in other comparative studies (RCT or propensity-matched score studies).[105,112,114,142] The authors report a savings in antibiotics (excluding cost of the test) of €764 per patient (from a reduction in the number of antibiotics used per patient of 0.9). The authors do not provide the name or duration of the antibiotics used in the study. It should be noted that in England, antibiotics are typically given over a 5-7 day course with a daily cost around £50. The failure rate of the SeptiFast test was not considered.

Table 15: Comparison of baseline characteristics between the control and intervention group (adaptation of Table 2 in Alvarez *et al*, 2012[91])

	Control group (n=54)	Intervention (n=48)
Age, mean ±SD	65±14.7	54±12.9
Gender	83.30%	72.90%
Initial diagnoses		
- *Emergency abdominal surgery*	20.37%	18.75%
- *Elective abdominal surgery*	3.70%	4.17%
- *Pneumonia*	7.41%	-
- *Pancreatitis*	1.85%	14.58%
- *CNS lesions*	16.67%	10.42%
- *Polytrauma/head injuries*	7.41%	41.67%
- *Heart surgery*	37.04%	4.17%
- *Major vascular surgery*	5.56%	2.08%
- *Pneumonectomy*	-	2.08%

The second cost-minimisation study by Mancini *et al.,* (2014)[105] evaluated the use of SeptiFast versus blood culture and is based on a propensity score-matched approach with 101 matched SIRS-SS episodes. The authors reported a trend (although not significant) in the reduction in SIRS-SS-related mortality associated with SeptiFast. The authors reported that the differences in mortality reached statistical significance when the tolerance (calliper) used for the propensity matching was reduced to only 5%, corresponding to 77 matched episodes (64 patients in the intervention group and 68 in the control group). Although the use of a lower calliper improved the quality of matching, the use of a lower calliper when matching reduced the precision by decreasing the sample size but could also introduce bias to the estimate as it is no longer the effect of treatment in the treated subjects that is being estimated, but the effect of treatment in those treated subjects for whom a control was found.[143] Using the tight calliper of 0.05, the authors showed a mortality rate of 3.13% for the intervention group and 14.71% for the control group (i.e. a difference in the mortality rate of 11.58%). Based on a pilot study, the authors reported that 278 pairs of SIRS event were needed to demonstrate a 10.6% difference in mortality (9.7% vs. 20.3%) which is greater than the numbers analysed in Mancini *et al*.[105] Results from this study therefore need to be interpreted with caution. It should also be noted that the PCR test was implemented under optimal condition. The authors showed no reduction in SIRS-SS episode length; even under a tight strict matching criterion. In this study, the SeptiFast test was estimated to lead to a reduction in medication. Based on information provided in a supplementary

appendix associated with the paper, patients receiving SeptiFast had a higher cost of antibiotics (€11.78). In contrast, patients had a reduction in antimycotics (commonly termed as antifungals in England) medication costs (€376.62), antiviral agents (€1.65) and other drugs (€91.39). Although the two cohorts were propensity matched it is unclear whether the suspected organisms in the two cohorts were matched which may affect the antibiotics prescribed. In the control arm, antimycotics represented about 53% of total medication costs. This figure appears relatively high compared with that expected in England based on Warhurst *et al*.[10] It is also unclear how the cost of antibiotics and antifungals differ between England and Italy. The failure rate of the SeptiFast test was not considered nor was it clear whether patients were randomly selected to receive SeptiFast or whether these were selected based on their characteristics.

Finally, the Bilkovski study (2014)[130] is a decision tree using evidence from the RADICAL study[121] on the impact of the IRIDICA-PLEX-ID hybrid assay (referred to by the authors as the PCR/ESI-MS) in term of treatment decision and studies on MALDI-TOF MS[125,126] for the impact of the test on clinical outcomes. This study was only available as a poster presentation; therefore it is difficult to judge the quality of this study. However, the External Assessment Group notes some inconsistencies. [redacted] However, the economic evaluation conducted by Bilkovski *et al.,* (2014)[130] is based on 422 patients and includes only 290 patients surviving to hospital discharge. Patients that are tested positive using IRIDICA-PLEX-ID hybrid were assumed to experience a reduction in hospital and ICU length of stay due to earlier availability of results and potential therapy changes based on data from two previous studies of MALDI-TOF MS.[125,126] The validity of this assumption is uncertain as MALDI-TOF MS and the IRIDICA-PLEX-ID hybrid assay are used differently and provide different information. Notably, studies of MALDI-TOF MS are conducted in patients with a positive blood culture only. Therefore the impact of treatment changes when blood culture is negative is unknown and may be associated with unintended consequences. Importantly, studies on MALDI-TOF MS[125,126] used in the economic evaluation evaluated the impact of MALDI-TOF MS in combination with an anti-stewardship programme compared with blood culture prior to the program. Hence, the impacts of MALDI-TOF MS in these studies are highly likely to be confounded by the simultaneous introduction of an anti-stewardship programme. These studies were also non-randomised in nature and therefore subject to potential biases. Bilkovski *et al.,* (2014)[130] predicted that the use of the IRIDICA-PLEX-ID hybrid system would lead to a reduction in hospital and ICU length of stay of 1.6 days and 0.7 days in all patients and 4.2 and 1.8 days in patients with positive IRIDICA-PLEX-ID results, respectively. It is unclear how the model estimate relates to the impact of the test in practice and whether such savings would be observed. As previously stated, evidence from five comparative studies of SeptiFast on the impact of a similar test in terms of

mortality or ICU and hospital length of stay is inconclusive.[91,102,105,112,114] Test failure was not considered in the analysis.

3.2 Relevance of existing economic evaluations to NICE decision making

Overall, the existing economic evidence has limited relevance to the current UK setting. To date, only two of the three tests (SeptiFast and IRIDICA-PLEX-ID hybrid) have supporting published economic evidence. Howeve, a number of limitations are noted.

There were a number of issues in the evaluations that require further consideration:

- It is unclear if results are generalisable to the UK. Notably, the current standard of care, the type of antibiotics used, and costs may differ between countries.
- All economic evaluations compared the use of either SeptiFast or the IRIDICA-PLEX-ID hybrid test against blood culture. No comparison is provided against MALDI-TOF MS; which is an increasing part of current practice in some units in the UK.
- Two economic evaluations were conducted within studies.[91,105] However, there are limitations due to the retrospective nature of these studies and potential biases associated with patient selection. Notably, the study by Alvarez *et al.,* (2012)[91] is believed to be highly confounded with large imbalances between groups in terms of initial diagnosis as 20 patients had heart surgery and 4 poly-trauma / head injuries in the control group of 54 patients compared with 2 patients following heart surgery and 20 poly-trauma / head injuries in the SeptiFast group of 48 patients.
- Two studies used modelling approaches combining evidence on the impact of SeptiFast[131] or the IRIDICA-PLEX-ID hybrid assay[130] against blood culture on treatment modification, with the impact associated with inadequate therapy or rapid identification. The results produced are contrary to the evidence of the impact of any of the tests on clinical outcomes. At present, evidence of the impact of any test on the outcomes of mortality and hospital/ICU length of stay is inconclusive from five comparative studies comparing SeptiFast against blood culture, with or without, MALDI-TOF MS.[91,102,105,112,114] In the SeptiFast modelling study[131] evidence on the impact of the test on treatment change and impact on clinical outcomes was based on relatively old evidence that was collected prior to recent guidelines on the diagnosis and treatment of sepsis.[16,18-20] In the IRIDICA-PLEX-ID hybrid assay modelling study, Bilkovski *et al*., (2015)[130] used data from two MALDI-TOF MS studies to derive the benefit associated with earlier results.[125,126] There are limitations as the studies were non-randomised in nature and therefore subject to biases and evaluated the use of MALDI-TOF MS in combination with an antistewardship programme in positive blood culture only.

Furthermore, the potential impact of the test on treatment modification is estimated retrospectively which may introduce biases.

- The cost of SeptiFast differed between the three studies[91,105,131] and do not necessarily reflect the most likely cost estimated by the External Assessment Group. Similarly, the cost of the IRIDICA-PLEX-ID hybrid assay[130] does not necessarily reflect the most likely cost estimated by the External Assessment Group.
- The range of costs included varied between studies. With the exception of Mancini *et al*., (2014[105]), most savings are attributable to a reduction in length of hospital stay. However, as previously mentioned, there is no robust evidence on the impact of any of the interventions on hospital or ICU length of stay.[91,102,105,112,114]
- Mancini *et al.,* (2014)[105] reported a reduction in the costs associated with 'classical diagnostic assays and instrumental procedures' following the introduction of SeptiFast and a large reduction in the costs of empirical therapy predominantly associated with anti-fungals. It is unclear whether these reductions are generalisable to the NHS in England.
- The identified economic evaluations focussed on the positive impact of the test, for example, a potential reduction in antibiotics and/or length of stay. It is noted that tests may be associated with unintended detrimental effects, such as a greater incidence of superinfection as reported in Leone *et al.,*[35] which evaluated the effects of de-escalation in patients with sepsis. Furthermore, any detrimental effects that may occur due to the wrong decision being made have been ignored.
- The results are also likely to be optimistic as the interventions appear to be implemented under optimal condition in the majority of identified economic evaluations[105,131] rather than considering delays that may occur under current practice conditions.
- The benefits associated with better antibiotic stewardship are not included in the economic evaluations however, the External Assessment Group acknowledge the difficulty of robustly quantifying such benefits.
- It is also unclear whether patients were pre-selected in the studies,
- Finally, the failure rates within interventions were not considered. A recent UK study conducted by Warhurst, *et al*., (2015)[10] suggests a test failure rate of 7% for SeptiFast. An alternative study reported failure rates of 22.9%.[101]

3.3 Independent economic assessment – conceptual model and methods

The conceptual model developed by the External Assessment Group was relatively simplistic due to the lack of appropriate data. A decision tree approach was adopted with a lifetime horizon and discounting undertaken at 3.5% per annum.

The NICE diagnostic reference case[144] requests that cost effectiveness is presented in terms of cost per QALY gained. This has been adhered to although the authors highlight the considerable uncertainty in any estimate due to the lack of robust data on key components of the calculation.

The cost per QALY can be divided into the incremental costs incurred and the incremental QALYs gained.
The incremental costs should consider:

- The cost of each test / comparator
- The net effect on hospital length of stay for both ICU and non-ICU noting that rapid tests could be detrimental to the patient as well as beneficial
- The net effect on the costs of antimicrobial treatment
- Any net cost impact associated with the potential impact on antimicrobial resistance.

The incremental QALYs would ideally consider:

- The impact on sepsis-related mortality
- The impact of net effect on hospital length of stay for both ICU and non-ICU noting that rapid tests could be detrimental to the patient as well as beneficial
- Any net QALY impact associated with the potential impact on antimicrobial resistance.

Whilst the costs of the tests and comparators can be estimated relatively well from current data there are no conclusive data on any other parameter that are listed in the bullet points above that was identified within the External Assessment Group's review. Therefore a scenario analysis was undertaken in which these values were estimated by clinical experts.

Within the model it was assumed that the rapid identification of a pathogen could result in changes in four key outcomes. These were: 30-day mortality rates; the length of stay in an ICU; the length of stay in the hospital; and the costs associated with antimicrobial treatment. Of these, changes in the mortality rate were assumed to affect QALYs only, with the remaining categories assumed to affect costs only. This is a simplification in that, for example, additional time in an ICU may be associated with slightly lower QALYs, but the impact of such omissions was assumed not to affect the overall conclusions. In all scenarios the potential impact of better antimicrobial stewardship in terms of drug resistance was not evaluated due to both the complexity of such a task and the absence of information on how the tests would reduce antimicrobial use.

It was assumed that negative tests would not impact on any of the four key outcomes. This assumption was supported by the clinical experts to the External Assessment Group. The decision to ignore negative tests was due to the potential fatal consequences if treatment was withdrawn from a patient with sepsis. Acknowledged reasons for a false negative result include the pathogen being unable to be detected by the test or if the quantity of the pathogen was below the test's limit of detection. Similarly tests which would be denoted as failures were assumed to have no impact on the four key outcomes. Both negative tests and failures would, however, be associated with the cost of the test.

A pictorial representation of the conceptual model is provided in Figure 11. The net cost impact and the net QALY impact of rapid identification were used to estimate a cost per QALY gained ratio.

The evaluations presented by the External Assessment Group have been divided into five categories.

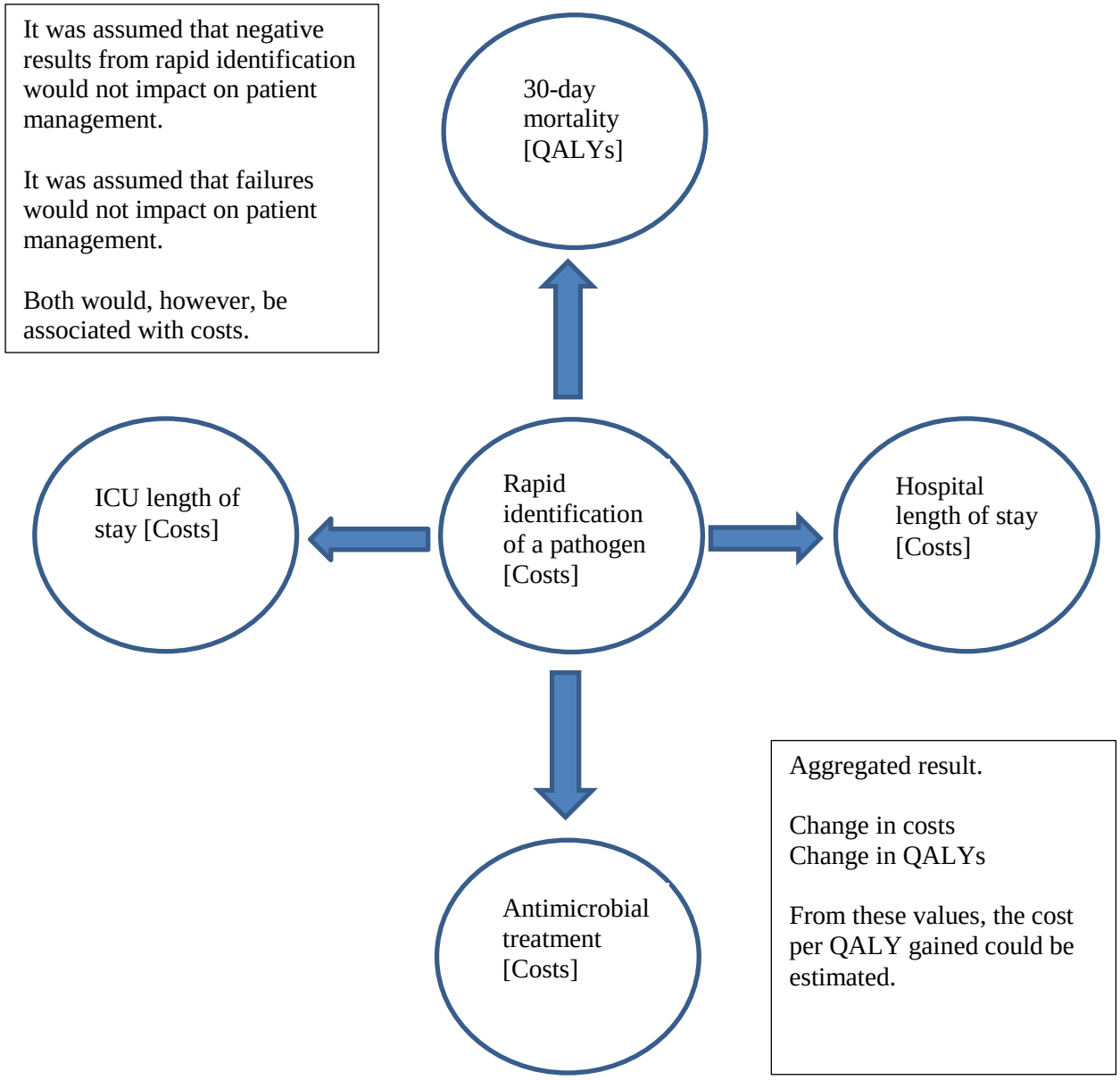
1) Base case 1: an analysis based on currently published evidence.
2) Base case 2: an analysis where parameter values were populated by estimates from clinical experts in order to estimate the cost effectiveness of each test. This has a benefit in that if, in Base case 1, the rapid tests offered little or no benefit compared with the comparators, based on the absence, or lack of statistical significance, of the required data then clinical beliefs could be incorporated.
3) Threshold analyses were undertaken to guide decision makers on the likelihood of the interventions having a cost per QALY gained values of £20,000 or lower and of £30,000 or lower as it was assumed that experts in the field would be more confident in providing an indication of whether the value of a parameter was greater than, or less than, a threshold number than in estimating a number in the absence of data (as was requested in Category 2). The variables assessed within the threshold analysis in the threshold base case were the number of mortalities within 30 days that were prevented and the reduction in days in ICU. For simplicity, and to allow thresholds to be presented purely in terms of net 30-day mortality or net cost, it was assumed that there was no additional QALY gain associated with a reduction in ICU duration of stay. Results are presented allowing for a mixture of both net mortalities and of net reduced ICU stay. In an alternate analysis the thresholds of both the reduction in the net number of ICU days and the net reduced costs of antimicrobial treatment are also presented.
4) Analyses comparing the interventions versus MALDI-TOF MS based on published literature
5) Analyses of data taken from studies where more than one intervention were compared directly

Given the large divergence in results produced by Base case 1 and Base case 2 the External Assessment Group decided that probabilistic sensitivity analyses would provide spurious accuracy in

relation with respect to the decision being undertaken. As such, only deterministic answers have been provided. If robust data are produced in relation to the efficacy of the interventions on key patient outcomes then probabilistic sensitivity analyses should be conducted.

The lack of evidence for heterogeneous diagnostic accuracy amongst subgroups resulted in the External Assessment Group only providing an overall measurement of cost effectiveness rather than by subgroup. Whilst the cost effectiveness may differ amongst subgroups, for example, a neonate would be expected to accrue more QALYs than an adult; these do not affect the fundamental uncertainty of whether the interventions would be associated with any key patient outcome.

Figure 11: The components within the conceptual model



For all but the threshold analyses the incremental cost per test has been calculated accounting for the net effect on ICU and hospital length of stay and changes in the costs of antimicrobial treatment. The rate of positivity for each test must also be known as it has been assumed that only positive intervention tests would result in a change in management.

As an illustrative example assuming that: the cost of a test was £400; each positive test was associated with a 0.1 days' reduction in ICU length of stay, 0.3 days' reduction in hospital stay, and a £50 reduction in antimicrobial treatment; and a 20% rate of positivity: the incremental costs would be estimated to be:

£400 + [-0.1 x £1057 + (-0.3 – -0.1) x £275 + -£50] x 20% = £357.86

The incremental QALYs have been calculated assuming 11.32 discounted QALYs per 30-day mortality avoided (see Section 3.4.1.4). Thus, if an intervention was assumed to reduce 0.01 deaths per test then the discounted QALYs gained would be 0.1132. To calculate the numbers of deaths avoided data are required on: the assumed underlying mortality rate at 30 days; the estimated reduction in the rate of 30-day mortality associated with each test; and the rate of positivity. As an example, if it was assumed that: the underlying mortality rate was 13%; the assumed reduction following a positive test was 5%; and the rate of positivity was 20% then it would be estimated that the number of deaths prevented would be 13% x 5% x 20% per test which equals 0.0013.

The principles outlined above in calculating incremental costs and QALYs have been maintained throughout the analyses undertaken in this report. For simplicity, the example provided above did not distinguish between the assumed impacts of the interventions when a subsequent blood culture was either negative or positive, although as detailed in Section 3.4.2 separate values for these were provided by the clinical experts.

3.4 Independent economic assessment – model population

3.4.1 General model parameters

3.4.1.1 The number of blood samples that need analysing per day

Three broad scenarios were undertaken regarding the number of blood samples that need analysing per day: based on the number observed in a recent clinical study[10] assuming an increase for community acquired infection (2.4); assuming 17; and assuming 68. These values were thought to provide a wide range of plausible values that could be undertaken within units of different sizes.

3.4.1.1.1 Assuming the numbers of samples that need analysing based on the number observed in a recent clinical study

These data were calculated from data reported in Warhurst *et al.*[10] as summarised in Table 16. This source was chosen as it was a recent, high-quality study set in England. In this study there was a central hub, in Salford, with a SeptiFast machine which supplied results to four sites. A monthly rate per site was calculated and then these were added to estimate a monthly throughput. This was assumed to be plausibly representative of the use of a centrally located machine relevant to an intervention serving a number of satellite hospitals. Note that the daily value does not equal the average across the Warhurst *et al.*[10] study due to the different lengths of enrolment in the study by site. It is not reported why the monthly rate in Site 4 was considerably lower than the remaining sites.

Table 16: Calculation of the estimate of the daily number of tests undertaken

	Number of months in study by Warhurst *et al.*,[10]	Number of tests sent from the site for analysis	Estimated tests per month
Site 1	30	481	16.03
Site 2	30	343	11.43
Site 3	21	170	8.10
Site 4	13	12	0.92
Total		1006	36.48

The summation of these sites' requirements was 36.48 tests per month although results were only provided for 922 out of 1006 samples, primarily due to a failure rate of 69/1006 (7%) for SeptiFast although 15 further samples were lost due to clinical reasons. The Warhurst *et al.*, study[10] only included healthcare-acquired sepsis. Based on clinical opinion it was assumed that 50% of sepsis cases were healthcare-acquired and 50% community-acquired and thus to include both types of sepsis the number of cases were doubled to 72.96 tests per month. This equates to 2.40 tests per day and it was assumed that on 60% of days two tests would be analysed and that on 40% of days three tests would be analysed. On clinical advice it was assumed that in this scenario an intervention would only be used 5 days a week and thus the numbers of tests were divided so that there was three times the expected number of tests on Monday compared with Tuesday to Friday. It was assumed that all tests sent for processing would be tested and that no allowance would be made for the possibility of rejecting requests for tests where the blood was sampled on a Friday evening but would not be processed until Monday morning. This assumption was supported by our clinical advisors who deemed that this would be how the system would work in practice, although clearly the gain in speed of identification is reduced for those samples collected on Friday evening and over the weekend. It

was assumed that there would be two runs on a Monday with an initial run analysing those samples collected after staff had finished working on a Friday evening, and a second run analysing those samples collected on the Monday itself.

3.4.1.1.2 Assuming the numbers of samples that need analysing were 17 per day

In this example the value was set to approximately seven times that estimated from the Warhurst *et al.,* study.[10] This value is also the maximum number of blood samples that one IRIDICA system can process in one day, assuming 3 runs per day, although it is noted that SeptiFast could process 21 tests over 3 runs and thus the selected number may favour IRIDICA. In this scenario it is assumed that practice has been changed to accommodate the interventions being used seven days a week, and that each intervention could be run three times daily. The costs of these changes in standard practice have not been incorporated, although a statement of the magnitude of this compared with any potential savings has been made.

3.4.1.1.3 Assuming the numbers of samples that need analysing were 68 per day

In this example the value was set to approximately twenty eight times that estimated from the Warhurst *et al.,* study.[10] This scenario assumes that four SeptiFast machines and four IRIDICA machines would be required to process the requested blood samples. In this scenario it is assumed that practice has been changed to accommodate the interventions being used seven days a week and that each intervention could be run three times daily. The costs of these changes in standard practice have not been incorporated, although a statement of the magnitude of this compared with any potential savings has been made.

3.4.1.2 The estimated costs of the interventions and MALDI-TOF MS

For all tests there is a fixed cost and a variable cost that is determined by the numbers of tests undertaken. Following advice obtained from our clinical advisors we assumed that the purchase cost of a machine could be equally divided over an assumed seven year usage period. Annual costs associated with the maintenance of the machines were incorporated.

For each intervention the cost will be dependent on whether the equipment required for the specific test (SeptiFast, SepsiTest, IRIDICA and MALDI-TOF MS) is already available within the microbiology laboratory. SeptiFast and IRIDICA have their own bespoke PCR machines, whereas SepsiTest can be run on a generic PCR machine. This led to two scenarios being evaluated for each intervention. These in order of cost are: 1) purchasing of the required machine for the specific test or a generic PCR machine; and 2) no additional machinery needing to be purchased.

The estimation of the cost of an average test is far from robust relying on uncertain assumptions in the number of tests per day and machinery costs and therefore adds additional uncertainty.

Note that in accordance with the NICE reference case for diagnostic evaluations that value added tax (VAT) is not included within economic evaluations.[144] Based on advice from our clinical experts it was assumed that no additional staff costs or room costs would be incurred if any of the interventions were purchased, although this is less likely to be the case should it be assumed that 17 or 68 samples need to be analysed per day. For simplicity, neither transport costs associated with sample testing nor additional staff training was included.

No discounts associated with bulk purchasing of equipment has been assumed for any intervention. It is possible that these exist in reality, if so the ICERs calculated for each intervention when large volumes of blood samples are analysed are likely to be over-estimates.

3.4.1.2.1 The costs associated with SeptiFast

Data provided by Roche Diagnostics on the list prices of the required Roche instruments to run a SeptiFast test is shown in Table 17. The sum of these individual items is £26,397 excluding VAT.

Additionally, Roche Diagnostics report an annual service charge of £3000 excluding VAT which covers any repairs required (including a replacement system if necessary) and an annual calibration check.

Table 17: The instruments required to run SeptiFast and their list prices

Instrument	Cat. No.	List Price
LightCycler® 2.0 Instrument with Software 4.1	03 531 414 001	£18,000.00
LC Carousel Centrifuge 2.0	03 709 582 001	£3,060.00
MagNALyser Instrument (230 V)	03 358 976 001	£4,500.00
Multi-Colour Compensation Set	4484355001	£536.76
SeptiFast Cooling Block	4555864001	£300.45

The key consumables required to run a test, along with their list price is provided in Table 18.

Table 18: The key consumables required to run a SeptiFast test, along with their list price

Product Name	Cat. No.	Roche List Price	Number per item
LightCycler M Grade Capillaries	3612066001	£ 883.93	768
LightCycler SeptiFast Kit CE	4469046001	£ 1,422.62	54
LightCycler SeptiFast meCA Kit, CE	4488814001	£ 624.44	10
SeptiFast Lys Kit, CE	4404432001	£ 745.95	200
SeptiFast Prep Kit, CE	4404459001	£ 320.73	10

Roche Diagnostics estimated the cost per test based on the number of tests per run, assuming one daily run and a five day working week. Running more tests per run reduces the average reagent cost per sample due to the requirement of having two control samples for each run. The costs estimated by Roche Diagnostics, excluding VAT for reagent costs are replicated in Table 19. From Table 21 it can be calculated that the marginal cost of one additional sample within a run is £122.00.

Table 19: Reagent cost per SeptiFast test estimated by Roche Diagnostics

Tests per run	Reagent cost per reportable result
1	£333.99
2	£228.00
3	£192.68
4	£175.01
5	£164.41
6	£157.35
7	£152.30

The estimated average costs per SeptiFast test are provided in Table 20 for the three scenarios related to the number of blood samples to be analysed.

Table 20: Estimated average costs per SeptiFast test

	Average cost per test assuming samples per day calculated from Warhurst *et al*.(2015)[10]	Average cost per test assuming 17 samples per day	Average cost per test assuming 68 samples per day
Assuming that SeptiFast machinery needs to be purchased	£205.54	£160.52	£154.28
Assuming no additional machinery is needed	£201.23	£159.91	£153.67

Assuming that the intervention costs were spread over a seven year period.

3.4.1.2.2 The costs associated with SepsiTest

As previously detailed (Section 1.7.2) an updated version of SepsiTest was recently released. This version does not require duplicate samples and thus the cost of the reaction per sample is halved. Due to the lack of diagnostic accuracy data on the updated version of the test, the analysis conducted in this report is based on the previous version of SepsiTest.

The company (Molzym Molecular Diagnostics) that manufactures SepsiTest report that the list price for 48 reactions for use in SepsiTest is £3000 including VAT which equates to £2500 excluding VAT, and a cost per reaction of £52.08, excluding VAT. Each test is assumed to require two slots amongst a 96 well PCR machine, and that two controls (one negative and one positive) are required for each run. Thus a maximum of 47 SepsiTest analyses can be performed within one run. The costs of the controls were not provided by Molzym: these were estimated to be £104.17 for the pair, which is the cost of 2 reactions. This is likely to underestimate the cost as additional costs associated with spiking blood to produce the positive control has not been included, although this omission is unlikely to affect the conclusions of the report.

Costs for additional sequencing following a SepsiTest positive test were assumed to be €11 for bacteria and €5.50 for fungi based on information supplied by Molzym Molecular Diagnostics. These values included VAT at 19% and were €9.24 and €4.62 respectively without VAT. Assuming an exchange rate of €1 to £0.72[139] these values were calculated as £6.66 and £3.33.

The costs of the machinery required to undertake PCR testing and Sanger sequencing was estimated from data provided by Molzym Molecular Diagnostics. These costs are reproduced in Table 19. The data was assessed by a clinical expert advising the External Assessment Group and was deemed appropriate. Therefore it was assumed that a cost of €60,000 (a high estimate based on the values in Table 19) would not be unreasonable to purchase the equipment to undertake PCR and include any maintenance required. This value is equal to £43,200 assuming an exchange rate of €1 to £0.72.[139]

Table 21: The costs assumed for undertaking the PCR required by SepsiTest as submitted by Molzym Molecular Diagnostics

Apparatuses	**Price**
• Thermomixer (24 x 2.0 ml tubes, adjust at 37ºC ,56ºC ,70ºC)	≈ 2,000. - €
• Cooling racks for 1.5 ml tubes (adjust at 4ºC,-20ºC)	≈ 50.- €
• Vortexer, e.g. VWR, Germany	≈ 170.- €
• Bench top microcentrifuge (≥ 13.000 rpm, ≥ 12.000 x g)	≈ 1,100.- €
• Clear work places: o UV workstation or o UV laminar flow, e.g. BDK UVF, Germany (optional)	 ≈ 3,000.- € ≈ 8,000.- €
• 1 set of precision pipettes: up to 10 µl, up to 20 µl, up to 200 µl and up to 1000 µl .e.g. Eppendorf, Germany	≈ 400.- €
• Thermocycler: o PCR cycler with o Gels analysis system or o Real-Time PCR cycler (ramp 2ºC /sec.)	 ≈ 3,000.- € ≈ 4000.- € ≈ 15,000.- €
• Sequencing analysis as service (GATC, Germany) • ABI 310 (Applied Biosystems), refurbished	≈ 5.- € per sequencing ≈ 30,000.- €
Plastic ware	
• Pipette tips with aerosol filter (e.g. Biosphere®,Sarstedt, Germany)	
• PCR tubes	
Chemicals	
• DNA decontamination: DNA Exitus®,Applichem, Germany (#A7089,0100),	
• Agarose gel (2%) for standard PCR	
• A container for plastic waste (pipette tips, vials, tubes) and another for liquid waste, autoclavable	
Others	
• Sterile disposables: o Gloves, e.g. Kimberly-Clark, Germany o Sleeves, e.g. Cardinal Health, Ireland o Bouffant Covers ,e.g. VWR, Germany o Overshoes, e.g. hygi, Germany	

DNA: Deoxyribonucleic acid; PCR Polymerase chain reaction; UV ultraviolet spectroscopy

In order that the profile of bacteria and fungi were representative of that observed in England an assumption was made that the proportion of positive results for bacteria and fungi identified by SeptiFast would be applicable to SepsiTest. This proportion, calculated from Table 14 of Warhurst *et al.*,[10] was 18 fungi from 167 positive SeptiFast tests (10.8%) which was used to estimate a weighted

cost of additional sequencing of £6.30. Based on the data synthesis conducted (Figure 6) it was assumed that SepsiTest had a sensitivity of 0.48 and a specificity of 0.86, which when combined with assumed blood culture positive rate of 8.7% calculated from Warhurst *et al*.[10] equated to a positivity rate of 17% for SepsiTest. The company manufacturing SepsiTest confirmed that the cost per SepsiTest assay was not dependent on throughput.

The estimated average costs per SepsiTest assay are provided in Table 22. These costs assume the sensitivity and specificity of SepsiTest as reported in the data synthesis. In alternate analyses when specific trial data are used these costs will change slightly as the number of sequencing tests needed will differ due to assumed alternative diagnostic accuracy data.

Table 22: Estimated average costs per SepsiTest

	Average cost per test assuming samples per day calculated from Warhurst *et al*.study[10]	Average cost per test assuming 17 samples per day	Average cost per test assuming 68 samples per day
Assuming a generic PCR machine and sequencer needs to be purchased	£149.53	£112.29	£108.55
Assuming no additional machinery is needed	£142.48	£111.36	£108.30

Assuming that the intervention costs were spread over a seven year period.

3.4.1.2.3 The costs associated with an IRIDICA test

Abbott Diagnostics report that the cost of the IRIDICA analyser is £268,000 excluding VAT, and that the cost of annual maintenance is £30,150 excluding VAT. The cost of an IRIDICA test is reported to be £174 excluding VAT. Following clarification the manufacturer provided costs in relation to the number of tests per day as a control test is required to be changed every 24 hours. These costs are summarised in Table 23. Abbott Diagnostics assumed that it would be possible to analyse 23 samples per day assuming that 1 control is used at the start of the day along with five samples, and that subsequent runs would not need a control and would analyse six samples simultaneously. The External Assessment Group comments that whilst it is technically possible to conduct four runs of slightly under six hours within a 24 hour period that this is unlikely to be possible in practice and

assumed that 17 tests per day represented the limit of an IRIDICA machine. The estimated average costs per IRIDICA test are provided in Table 24.

Table 23: The assumed cost per IRIDICA test based on different numbers of blood samples to be analysed

Number of samples to be analysed	Total cost per sample (£)	Number of samples to be analysed	Total cost per sample (£)
1	362.04	13	196.56
2	273.29	14	195.71
3	242.53	15	194.73
4	226.28	16	193.65
5	215.82	17	192.49
6	211.78	18	192.44
7	208.38	19	192.21
8	205.40	20	191.93
9	202.69	21	191.31
10	200.18	22	190.68
11	197.80	23	189.96
12	197.28		

Table 24: Estimated average costs per IRIDICA test

	Average cost per test assuming samples per day calculated from Warhurst *et al*.study[10]	Average cost per test assuming 17 samples per day	Average cost per test assuming 68 samples per day
Assuming the IRIDICA analyser needs to be purchased	£314.61	£203.52	£203.52
Assuming no additional machinery is needed	£270.89	£197.35	£197.35

Assuming that the intervention costs were spread over a seven year period.

3.4.1.2.4 The costs associated with a MALDI-TOF MS system

Costs associated with MALDI-TOF MS were provided by Bruker UK Limited (personal communication with Erika Tranfield, May 2015). It was assumed that these costs would be generalisable to other MALDI-TOF MS systems such as bioMérieux's VITEK® MS system. The cost of the MALDI-TOF MS machine was assumed to be £125,000. A further technology the Sepsityper kit, is available at a cost of approximately £3 per test. The Sepsityper kit is a sample preparation method that involves the lyses of blood cells, followed by centrifugation and washing steps to produce a pellet of bacteria or fungi, which is further processed by standard MALDI-TOF MS methods. Given that Sepsityper was in widespread use in the RAPIDO trial,[31] a large UK study comparing MALDI-TOF MS and standard practice with standard practice alone, it has been assumed that this will be used for the purposes of this economic evaluation. On clinical advice no further costs for MALDI-TOF MS were assumed in addition to that for the Sepsityper kit (assumed to be £3) as these were relatively inexpensive. The costs for preventative maintenance are dependent on the number of maintenances performed per year: for two maintenances per year the cost is £13,985 whereas the cost for 3 to 5 maintenances per year is £17,000. It was assumed that the higher number of preventative maintenances applied.

It is noted that MALDI-TOF MS can be used for many other investigations than just the use of pathogen identification in those with suspected sepsis. Based on clinical advice it was assumed that only 50% of the purchase and maintenance cost would be attributable to sepsis investigations. Our clinical advisors did not advocate attributing the costs of any of the interventions to non-sepsis related disease areas.

Data on the number of blood culture bottles that are flagged as positive were taken from Warhurst *et al.*,[10] and assumed generalisable to England. Data from Table 6 of Warhurst *et al.*,[10] show that of 922 episodes, 80 were blood culture positive (8.7%) and it was assumed that this percentage would receive further analysis via MALDI-TOF MS. As such, it was estimated that the MALDI-TOF MS machine would process 8.7% of the daily throughput of 2.40 tests (see Section 3.4.1.1.1) which is 0.21 tests per day. When the assumed number of blood samples needing analysing was increase to 17 and 68 per day the number of samples analysed using MALDI-TOF MS was increased to 1.48 and 5.92 per day respectively.

Care should be taken not to directly compare the costs per test between the interventions and MALDI-TOF MS since all samples would be processed by the interventions, whilst only those where blood culture had tested positive would be analysed by MALDI-TOF MS.

Table 25: Estimated average costs per MALDI-TOF MS

	Average cost per test assuming samples per day calculated from Warhurst *et al*. study[10]	Average cost per test assuming 17 samples per day	Average cost per test assuming 63 samples per day
Assuming a MALDI-TOF MS machine needs to be purchased	£232.39	£35.35	£11.09
Assuming no additional machinery is needed	£114.88	£18.78	£6.94

Assuming that the intervention costs were spread over a seven year period.

3.4.1.2.5 The costs associated with blood culture

Given that blood culture would be used alongside all interventions and alongside MALDI-TOF MS the costs would have no bearing on the incremental costs associated with the intervention tests and MALDI-TOF MS. For this reason no resources were spent in trying to ascertain the costs per blood sample and the cost was assumed to be £0 in all analyses.

3.4.1.3 The assumed failure rates of the interventions

Both SeptiFast and IRIDICA use internal controls which could be subject to failure as could the controls on a PCR machine used by SepsiTest. For SeptiFast a 6.9% failure rate was reported in Warhurst *et al.*,[10] although a greater value of 22.9% was reported in Paolucci *et al.*[101] Data on the failure rate of IRIDICA have been reported in Metzgar *et al*, [119] and indicate a rate of [redacted]. No data on the failure rate of SepsiTest were identified.

In the base case it has been assumed that SeptiFast has a failure rate of 6.9%. In sensitivity analyses a failure rate of 11.7% was assumed for SeptiFast based on a naïve pooling of failure results from Warhurst *et al.*[10] (69) and from Paolucci *et al*.[101] (100) divided by the numbers of samples analysed in all of the SeptiFast versus blood culture trials combined (11,659) assuming no failures in any SeptiFast study. This results in an estimated failure rate for SeptiFast of 1.4%. For the base case for IRIDICA a failure rate of [redacted] was assumed based on a naïve pooling of data from all of the studies assuming no failures in any study but Metzgar *et al*.[119]

Whilst Warhurst *et al.,*[10] Paolucci *et al*.[101] and Metzgar *et al,*[119] explicitly stated that any failures were excluded from analyses of diagnostic accuracy, it is not clear whether failures occurred in the remaining identified studies but were not reported as such and were treated as a negative result. If failures had been excluded from the analysis of diagnostic accuracy but not reported then this would be beneficial to the relevant intervention.

3.4.1.4 The QALY gains associated with preventing a 30-day mortality

It was assumed that each 30-day mortality avoided is associated with a gain of 11.32 discounted QALYs. This value was calculated based on (a) the estimated number of discounted life years for a typical patient and (b) the estimated quality of life after a sepsis episode to account for the possible reduced quality of life in sepsis survivors. It should be noted the discounted QALY gains for neonates and children would be greater than those for adults, due to the longer life expectancy although the exact increase is uncertain.

Whilst there is evidence that the survival after a sepsis episode may be lower compared with the general population[145-147] for simplicity, we assumed that patients with sepsis had a comparable survival to that of the general population. National life tables for England and Wales for the period 2011-2013[148] were used to estimate the life expectancy of a typical patient, assuming an age of 58 years and a gender split of 60%/40% as reported in Warhurst *et al.*[10]

Patients were assumed to have a utility value of 0.68 based on the Euro-Qol 5 dimensions score reported by Cuthbertson *et al.*[145] at 5 years after a severe sepsis episode, which is similar to the value reported by Drabinski *et al*.[149] If the utility predicted for the general population for an age and sex profile[150] was lower than 0.68 this value was used instead. A discount rate of 3.5% per annum was used as recommended in the NICE reference case.[144]

3.4.1.5 The assumed cost of a day's treatment within an ICU

This value was calculated from NHS reference costs.[151] Service code CCU03 (medical adult patients (unspecified specialty)) was assumed to be representative of treatment for sepsis patients. This service code is subdivided by the number of organs supported, ranging from 0 to 6 or more, and an average of the reported average unit costs weighted by activity levels was calculated. This resulted in a cost of ICU care of £1057 per day. This is slightly more than the weighted average were service code CCU002 (surgical adult patients (unspecified specialty)) selected which was £987 per day.

3.4.1.6 The assumed cost of a day's treatment within a standard hospital ward

This value was calculated from NHS reference costs,[151] assuming that the average excess bed day cost per non elective impatient of £275 was appropriate.

3.4.1.7 The assumed cost of typical empirical antimicrobial treatment for sepsis

Based on the advice of our clinical advisors it was assumed that 7 days' treatment with either 18g per day of piperacillin/tazobactam or 3g per day of meropenem was an appropriate empirical treatment for typical sepsis patients. Using BNF costs[152] these prices were estimated to be £51.60 a day (assuming 4.5g every 6 hours) for piperacillin/tazobactam and £48.00 (assuming 1g every 8 hours) for meropenem. Given the uncertain proportion of the drugs used in England it was assumed that a cost per day of £50, equating to a cost for a course of treatment of £350, was not unreasonable. However, an expert clinician on the Diagnostic Appraisal Committee commented that these costs may be high for adults admitted to regular hospital wards or for children. If this was the case then the value used for a course of treatment would be favourable to the interventions.

3.4.1.8 The assumed 30-day mortality rate for those with suspected sepsis

It was assumed that the 28-day mortality rate reported in a recent HTA set in England could be generalised to a 30-day mortality rate. This value was 13% (95% CI 11% to 16%)[10] with the rate of hospital mortality being 21% (95% CI 17% to 23%). This value has some support from data provided by Kaukonen *et al.*,[3] which analysed hospital mortality rates in patients with severe sepsis in Australia and New Zealand and showed a decrease across time with values of approximately 10% for SIRS-positive sepsis and 20% for SIRS-negative sepsis.

An alternate value of 29% as reported in Mouncey *et al.*,[9] (albeit for 90-day mortality) in patients with early septic shock was tested in sensitivity analyses. This value was supported by data from Levy *et al.*,[6] which reported hospital mortality rates of 29% where there was high compliance with a resuscitation bundle, although the patients included in this study were those with severe sepsis and septic shock and would be likely to have a worse prognosis.

3.4.2 Model parameters assumed for Base case 1

In Base case 1 only data from the published literature related to patient outcomes were included in an economic evaluation to estimate the cost-effectiveness of each test.

Based on the literature identified by the External Assessment Group no data were found that provided a conclusive and non-confounded indication that any of the interventions provided benefits in terms of: 30-day mortality; length of stay in ICU; or length of stay in hospital. One study of a propensity score-matched design was identified[105] that indicated a significant reduction in the costs of empirical therapy, but this had a number of limitations. Firstly the study population was haematological patients who were prescribed empirical antifungals which is not a typical suspected sepsis patient. Secondly, the cost savings predominantly came from the reduction in relatively expensive empiric antifungals;

however, according to our clinical experts the most widely used antifungal treatment in England is fluconazole which is now relatively cheap with a cost of £1.83 per day for a dose of 400mg.[152]

Data were found showing that the time to therapy modification was much quicker following the introduction of SeptiFast (18.8 hours compared with 38.3 hours)[114] but no data were provided on the change in costs of modified therapy. Changes in costs were also not provided for the studies identified in Table 9. As such, it was assumed that the costs of antimicrobials were unchanged in the base case.

Thus, in Base case 1, no difference was assumed in the results for when an intervention was used and when it was not used. An analysis was undertaken to assess the reduction in antimicrobial costs due to an intervention that would be required per test for each intervention in order to be cost neutral.

3.4.3 Model parameters assumed for Base case 2

In Base case 2 parameter values were populated using estimates from clinical experts in order to estimate the cost effectiveness of each test. A document (reproduced in Appendix 7) was sent to the clinicians on the Diagnostic Assessment Committee and to the clinical experts who are authors of this report with a request to estimate key parameters - supporting information identified by the External Assessment Group was also supplied. Seven clinical experts responded with a wide variation in the answers provided. Although the clinicians were asked for ranges in their answers six of the seven clinicians provided point estimates only. Typically the clinicians reported that the task was difficult to complete and the majority of clinicians assumed the same values for all three interventions, although not for MALDI-TOF MS, as the information came at a later time point than for the interventions. The average values from the clinical experts are provided in Table 26 for when an intervention was positive and the subsequent blood culture was positive, and in Table 27 for when an intervention was positive and the subsequent blood culture was negative. Note monetary savings in antimicrobial costs were transformed into a percentage reduction assuming a typical course of treatment cost £350.

The clinicians predicted comparable gains when the blood culture was negative with when the blood culture was positive. This is believed to be because the clinical experts trusted the intervention result rather than the blood culture result in this scenario. This contrasts with the majority of diagnostic accuracy studies where blood culture is assumed to be the gold standard and casts uncertainty over meta-analyses where this assumption is made.

Consideration was given to attempting to construct a distribution to represent the values provided by the clinicians although this was not undertaken as it was not clear that this would provide significantly better data than an aggregate value and analysis at the individual clinician level and further

assumption would need to be made regarding the distribution type and in estimating the range in the midpoint answers provided.

Table 26: The parameter values assumed in Base case 2 when the intervention was positive and the subsequent blood culture was positive

	SeptiFast	SepsiTest	IRIDICA	MALDI-TOF MS
Average net effect on ICU length of stay (days)	-0.607	-0.671	-0.736	-0.175
Average net effect on hospital length of stay (days)	-1.050	-1.214	-1.329	-0.758
Average net effect on the cost of antimicrobials	-17.78%	-21.63%	-25.92%	-14.26%
Net effect on 30-day mortality	-3.16%	-3.87%	-4.59%	-3.00%

Table 27: The parameters values assumed in Base case 2 when the intervention was positive and the subsequent blood culture was negative

	SeptiFast	SepsiTest	IRIDICA
Average net effect on ICU length of stay (days)	-0.571	-0.629	-0.700
Average net effect on hospital length of stay (days)	-1.307	-1.471	-1.586
Average net effect on the cost of antimicrobials	-28.98%	-31.84%	-37.12%
Net effect on 30-day mortality	-3.93%	-4.64%	-5.36%

Data from individual clinicians were used in sensitivity analyses. These values are shown in Tables 28 to 31 for the three interventions and MALDI-TOF MS.

Table 28: Individual clinician responses for SeptiFast when the subsequent blood culture was positive

	Average net effect on ICU length of stay (days)	Average net effect on hospital length of stay (days)	Average net effect on the cost of antimicrobials	Net effect on 30-day mortality
Clinician 1	-0.2	-0.5	-5%	-3%
Clinician 2	0	-1	-15%	-1%
Clinician 3	-0.1	-0.2	-£40 (-11%)	-0.1%
Clinician 4	-2.5 range (-4 to -1)	-1.5 range (-3 to -0)	Not known (assumed to be £0)	-1.0% range (-2% to -0%)
Clinician 5	-1	-3	-£175 (-50%)	-2%
Clinician 6	-0.001	0	-18%	0%
Clinician 7	-0.45	-1.15	-25%	-15%

Table 29: Individual clinician responses for SepsiTest when the subsequent blood culture was positive

	Average net effect on ICU length of stay (days)	Average net effect on hospital length of stay (days)	Average net effect on the cost of antimicrobials	Net effect on 30-day mortality
Clinician 1	-0.2	-0.5	-5%	-3%
Clinician 2	0	-1	-15%	-1%
Clinician 3	-0.1	-0.2	-£40 (-11%)	-0.1%
Clinician 4	-2.5 range (-4 to -1)	-1.5 range (-3 to -0)	Not known (assumed to be £0)	-1.0% range (-2% to -0%)
Clinician 5	-1	-3	-£175 (-50%)	-2%
Clinician 6	0	0	0%	0%
Clinician 7	-0.9	-2.3	-70%	-20%

Table 30: Individual clinician responses for IRIDICA when the subsequent blood culture was positive

	Average net effect on ICU length of stay (days)	Average net effect on hospital length of stay (days)	Average net effect on the cost of antimicrobials	Net effect on 30-day mortality
Clinician 1	-0.2	-0.5	-5%	-3%
Clinician 2	0	-1	-15%	-1%
Clinician 3	-0.1	-0.2	-£40 (-11%)	-0.1%
Clinician 4	-2.5 range (-4 to -1)	-1.5 range (-3 to -0)	Not known (assumed to be £0)	-1.0 range (-2 to -0)
Clinician 5	-1	-3	-£175 (-50%)	-2%
Clinician 6	-0.001	0	-20%	0%
Clinician 7	-1.35	-3.1	-80%	-25%

Table 31: Individual clinician responses for MALDI-TOF MS when the subsequent blood culture was positive

	Average net effect on ICU length of stay (days)	Average net effect on hospital length of stay (days)	Average net effect on the cost of antimicrobials	Net effect on 30-day mortality
Clinician 1	-0.1	-0.2	-2%	-1%
Clinician 2	0	-1	-15%	-1%
Clinician 3	No answer provided			
Clinician 4	0	0	0	0
Clinician 5	-0.5	-1.5	-£100 (-29%)	-1%
Clinician 6	0	0	-10%	0
Clinician 7	-0.45	-1.85	-30%	-15%

Table 32: Individual clinician responses for the SeptiFast when the subsequent blood culture was negative

	Average net effect on ICU length of stay (days)	Average net effect on hospital length of stay (days)	Average net effect on the cost of antimicrobials	Net effect on 30-day mortality
Clinician 1	-1	-1	-15%	-8%
Clinician 2	0	-1	-15%	-1%
Clinician 3	0	-1.5	-£80 (-23%)	0%
Clinician 4	-2.5 range (-4 to -1)	-3.5 range (-5 to -2)	Not known (assumed to be £0)	-3.5% range (-5% to -2%)
Clinician 5	0	-1	-£700 (-100%†)	-0%
Clinician 6	-0.05	0.0	-25%	0%
Clinician 7	-0.45	-1.15	-25%	-15%

† The percentage reduction was capped at 100%

Table 33: Individual clinician responses for SepsiTest when the subsequent blood culture was negative

	Average net effect on ICU length of stay (days)	Average net effect on hospital length of stay (days)	Average net effect on the cost of antimicrobials	Net effect on 30-day mortality
Clinician 1	-1	-1	-15%	-8%
Clinician 2	0	-1	-15%	-1%
Clinician 3	0	-1.5	-£80 (-23%)	0%
Clinician 4	-2.5 range (-4 to -1)	-3.5 range (-5 to -2)	Not known (assumed to be £0)	-3.5% range (-5 to -2)
Clinician 5	0	-1	-£700 (-100%†)	-0%
Clinician 6	0	0	0%	0%
Clinician 7	-0.9	-2.3	-70%	-20%

† The percentage reduction was capped at 100%

Table 34: Individual clinician responses for IRIDICA when the subsequent blood culture was negative

	Average net effect on ICU length of stay (days)	Average net effect on hospital length of stay (days)	Average net effect on the cost of antimicrobials	Net effect on 30-day mortality
Clinician 1	-1	-1	-15%	-8%
Clinician 2	0	-1	-15%	-1%
Clinician 3	0	-1.5	-£80 (-23%)	0%
Clinician 4	-2.5 range (-4 to -1)	-3.5 range (-5 to -2)	Not known (assumed to be £0)	-3.5 range (-5 to -2)
Clinician 5	0	-1	-£700 (-100%†)	-0%
Clinician 6	0.05	0	-27%	0%
Clinician 7	-1.35	-3.1	-80%	-25%

† The percentage reduction was capped at 100%

3.5 Independent economic model - results

As previously stated the results will be presented in 3 broad categories: Base case 1, using only data from the published literature considered appropriate have been included in an economic evaluation to estimate the cost-effectiveness of each test; Base case 2, where evidence on parameters were populated from estimates by clinical experts in order to estimate the cost effectiveness of each test; and a series of threshold analyses. For clarity threshold analyses indicate the value of a parameter at which the decision is likely to change. In the results presented in this report these values indicate the level above which the cost per QALY reduces to an assumed value (either £20,000 per QALY or £30,000 per QALY). In addition, supplementary analysis using studies deemed to provide additional information on head to head comparisons between interventions, or of an intervention with MALDI-TOF MS, have been undertaken. Given the results of the data synthesis which did not show a difference in diagnostic accuracy by subgroup only one set of analyses are presented. This is acknowledged to likely underestimate the gains in prevented mortality associated with neonates, but it was deemed that this would not affect the decision as the fundamental uncertainty in the changes on key patient outcomes provided by the interventions.

3.5.1 Results from Base case 1

The estimated costs and estimated QALYs where only the machinery required for the intervention need to be purchased are shown in Table 35 and the results where no additional machinery needs to be purchased are provided in Table 36. For brevity, these results are only presented for the cost per test

estimated using the number of blood samples per day from the Warhurst *et al.*[10] study: the conclusions remain the same at lower cost of tests due to the assumed lack of QALY gain. This conclusion is that all the tests are dominated, in that they are associated with an additional cost, but are assumed to provide no additional QALYs.

Table 35: Estimated cost per QALY when it is assumed that the machinery required for the intervention need to be purchased

	Incremental Cost per test compared with blood culture (£)	Incremental QALYs gained per test compared with blood culture	Cost per QALY gained compared with blood culture
SeptiFast	205.54	0.00	Dominated
SepsiTest	149.53	0.00	Dominated
IRIDICA	314.61	0.00	Dominated

Dominated denotes that an intervention is more expensive and does not provide additional QALYs

Table 36: Estimated cost per QALY when it is assumed that no additional machinery needs to be purchased

	Incremental Cost per test compared with blood culture (£)	Incremental QALYs gained per test compared with blood culture	Cost per QALY gained compared with blood culture
SeptiFast	201.23	0.00	Dominated
SepsiTest	142.48	0.00	Dominated
IRIDICA	270.89	0.00	Dominated

Dominated denotes that an intervention is more expensive and does not provide additional QALYs

An analysis was undertaken to assess the reduction in antimicrobial costs due to an intervention that would be required per test for each intervention in order to be cost neutral. These results are shown in Table 37 when machinery needs to be purchased and in Table 38 when no additional machinery is required.

Table 37: The reduction in antimicrobial costs due to an intervention that would be required per test for each intervention in order to be cost neutral when machinery needs to be purchased

	Required reduction in antimicrobial treatment costs assuming samples per day calculated from Warhurst *et al*.study[10]	Required reduction in antimicrobial treatment costs assuming 17 samples per day	Required reduction in antimicrobial treatment costs assuming 68 samples per day
SeptiFast	59%	46%	44%
SepsiTest	43%	32%	31%
IRIDICA	90%	58%	58%

Table 38: The reduction in antimicrobial costs due to an intervention that would be required per test for each intervention in order to be cost neutral when machinery does not need to be purchased

	Required reduction in antimicrobial treatment costs assuming samples per day calculated from Warhurst *et al*.study[10]	Required reduction in antimicrobial treatment costs assuming 17 samples per day	Required reduction in antimicrobial treatment costs assuming 68 samples per day
SeptiFast	57%	46%	44%
SepsiTest	41%	32%	31%
IRIDICA	77%	56%	56%

If it was assumed that reduction in antibiotic costs would only come following positive tests then, assuming the positivity rates calculated for each test based on the estimated sensitivity and specificity values then the costs of the tests could not be recouped from reduced antimicrobial treatment costs alone.

3.5.2 Results from Base case 2

Results are presented separately using the average value from all clinician responses and by individual clinician. There is also a differentiation of the results based on the assumed mortality rate associated with suspected sepsis. As clinicians provided estimates for the potential benefit of MALDI-TOF MS in addition to the interventions this technique has also been included in the tables. These results are divided into those assuming a mortality rate of 13% from Warhurst *et al.*,[10] and assuming 29% from Mouncey *et al*.[9] For SeptiFast the results from Warhurst *et al*.[10] have been given primacy as this is an English study[10] assessed as high quality, however, sensitivity analyses have been undertaken using the evidence from the data synthesis undertaken in this report. For SepsiTest and IRIDICA the results presented use the evidence from the data synthesis.

There are different combinations of machinery purchasing requirements dependent on the machinery in place in units that would process the blood samples. In order to facilitate an estimation of the relative cost effectiveness of each intervention and MALDI-TOF MS across these combinations the results have been summarised in terms of net monetary benefit (NMB^{153}) compared with blood culture. NMB can be compared simply with the strategy with the largest net benefit being estimated to be the most cost-effective. Twelve summary figures (Figures 12 to 23) are presented which are combinations of assumed mortality rate (either 13% or 29%) and maximum acceptable incremental cost effectiveness ratio (MAICER) (either £20,000 or £30,000 per QALY gained) and blood samples requiring evaluation per day (2.4, 17 or 68). It is likely that the mortality rate is inversely correlated to the number of tests per day, in that large throughput may be associated with a greater proportion of relatively minor investigations. This is noted as a limitation but has not been formally investigated. Following this summary' the results for each intervention in each scenario are presented reporting incremental QALYs, incremental costs and ICERs compared with blood culture. All results are presented assuming a timescale of testing of 1 year, although discounted QALYs accrued in future years are included.

It is seen that regardless of the scenario all three interventions and MALDI-TOF MS produced a positive net benefit compared with blood culture. This conclusion was not affected regardless of whether the acquisition cost of the machine was incorporated in the calculation.

In the scenario where the mortality rate is assumed to be 13%, the MAICER to be £20,000 per QALY and 2.4 blood samples per day, SepsiTest or IRIDICA has the highest estimated NMB dependent on assumption regarding machine purchase. For all other scenarios IRIDICA is estimated to have the highest NMB. However, these results are highly uncertain and it remains plausible that each (or none) of the interventions is most cost effective.

However, as will be detailed later, these results are highly variable by individual clinician questioned, and thus there is large uncertainty in these estimates.

Figure 12: NMB assuming a MAICER of £20,000, a mortality rate of 13% and 2.4 blood samples to be analysed per day

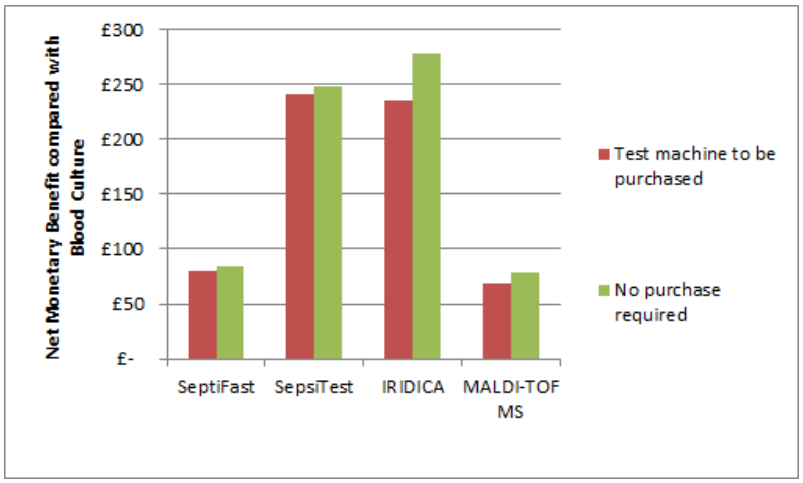


Figure 13: NMB assuming a MAICER of £30,000, a mortality rate of 13% and 2.4 blood samples to be analysed per day

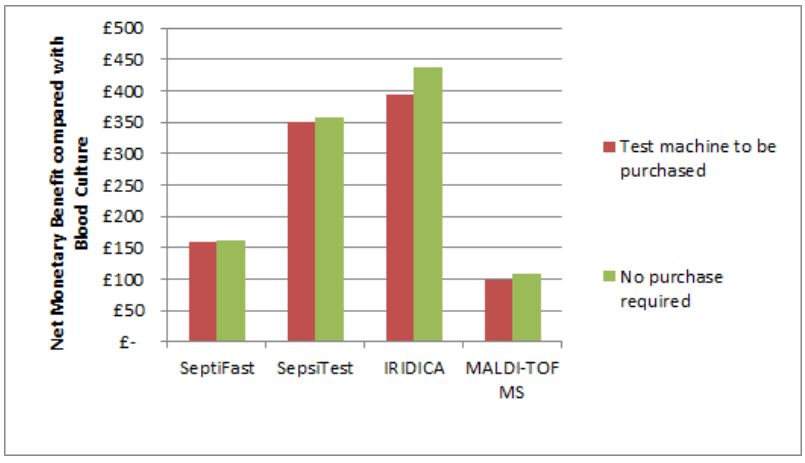


Figure 14: NMB assuming a MAICER of £20,000, a mortality rate of 29% and 2.4 blood samples to be analysed per day

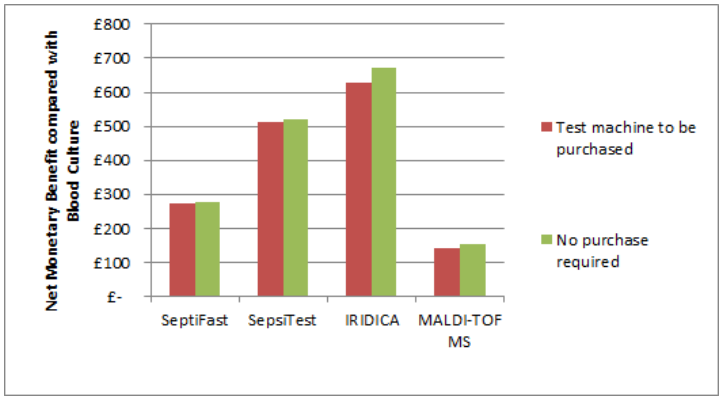


Figure 15: NMB assuming a MAICER of £30,000, a mortality rate of 29% and 2.4 blood samples to be analysed per day

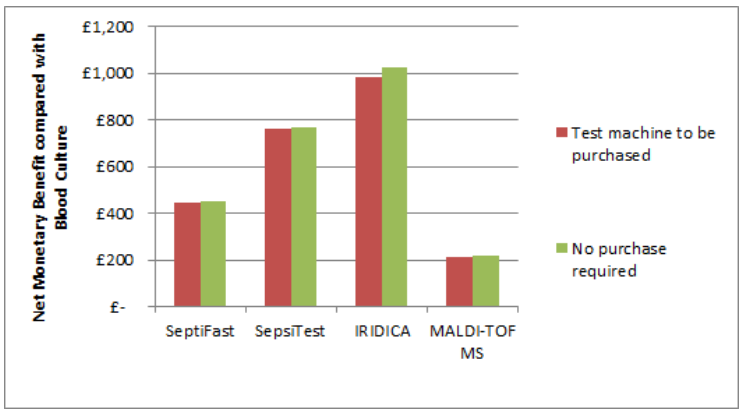


Figure 16: NMB assuming a MAICER of £20,000, a mortality rate of 13% and 17 blood samples to be analysed per day

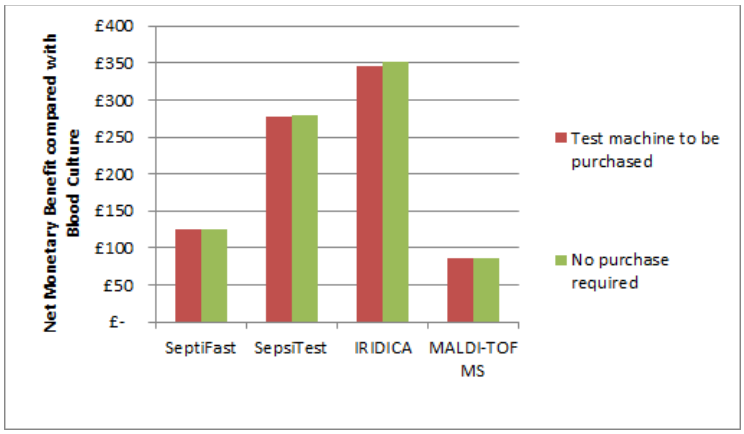


Figure 17: NMB assuming a MAICER of £30,000, a mortality rate of 13% and 17 blood samples to be analysed per day

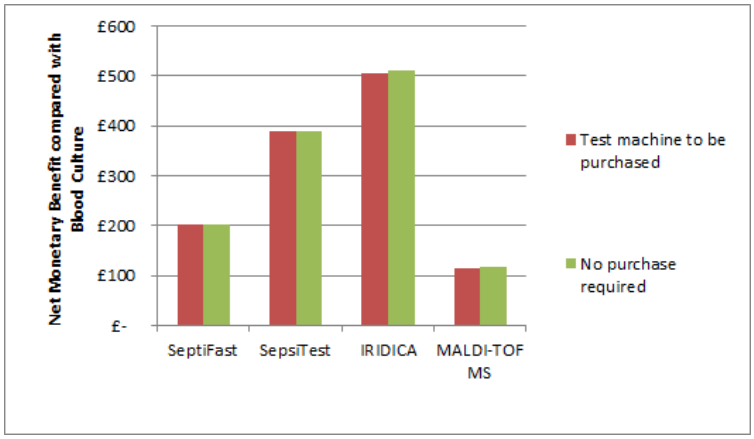


Figure 18: NMB assuming a MAICER of £20,000, a mortality rate of 29% and 17 blood samples to be analysed per day

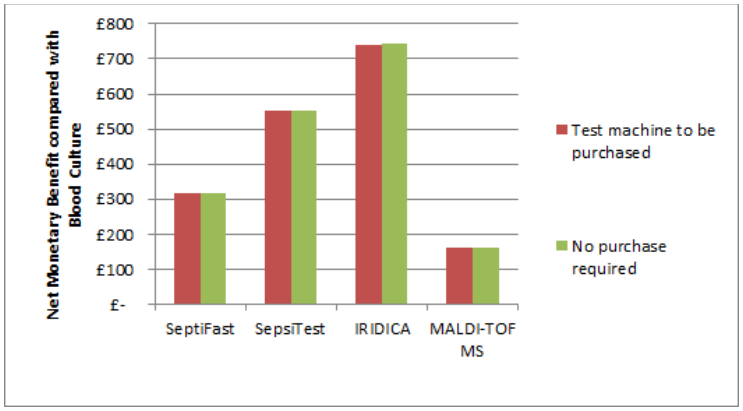


Figure 19: NMB assuming a MAICER of £30,000, a mortality rate of 29% and 17 blood samples to be analysed per day

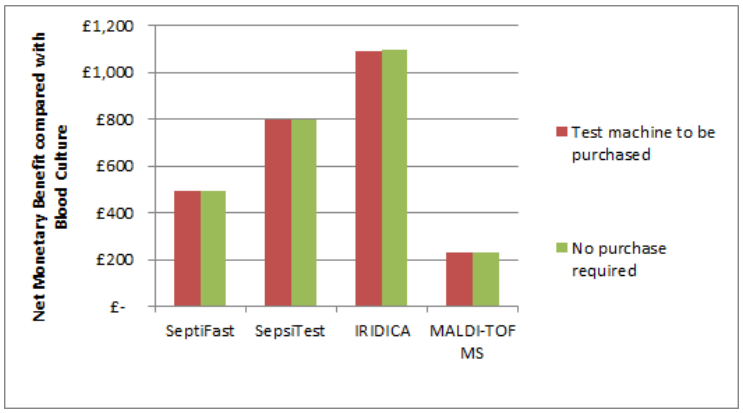


Figure 20: NMB assuming a MAICER of £20,000, a mortality rate of 13% and 68 blood samples to be analysed per day

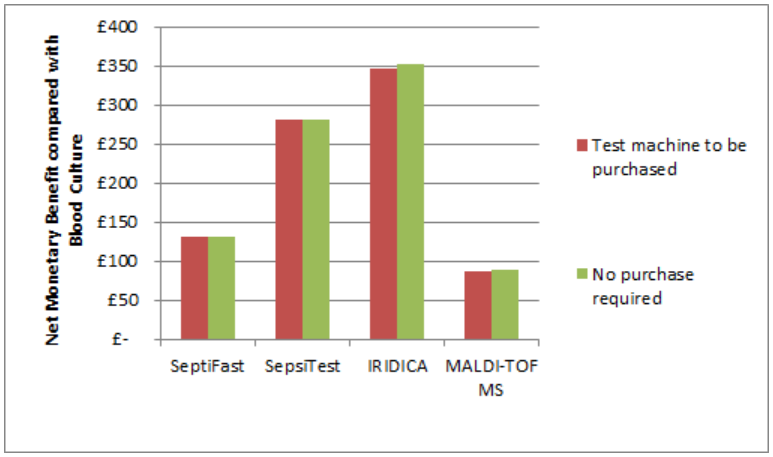


Figure 21: NMB assuming a MAICER of £30,000, a mortality rate of 13% and 68 blood samples to be analysed per day

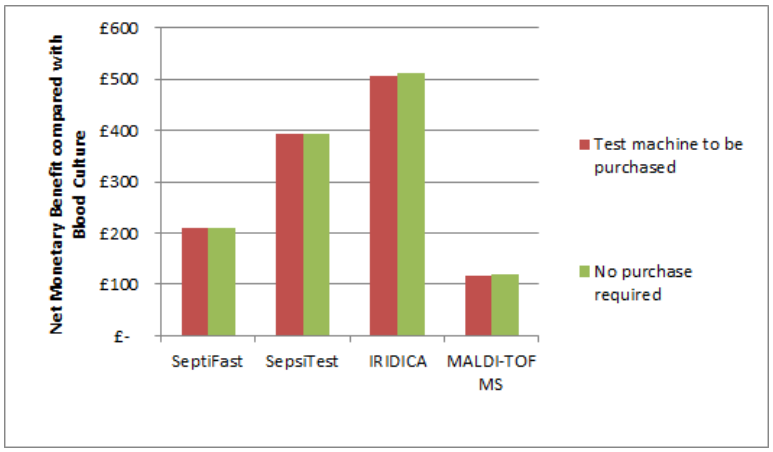


Figure 22: NMB assuming a MAICER of £20,000, a mortality rate of 29% and 68 blood samples to be analysed per day

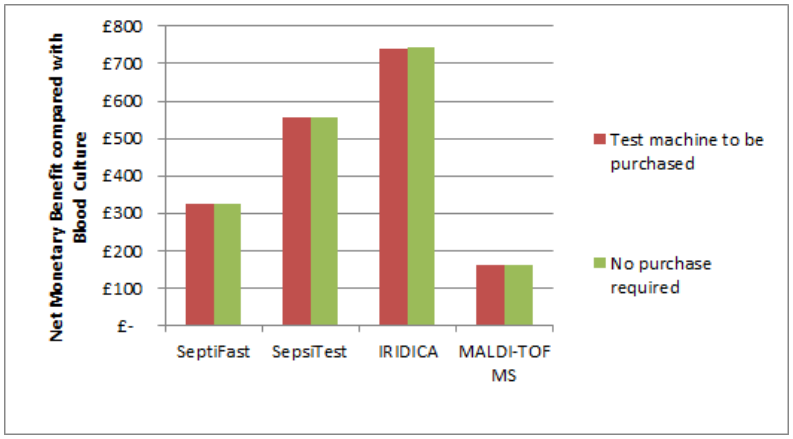
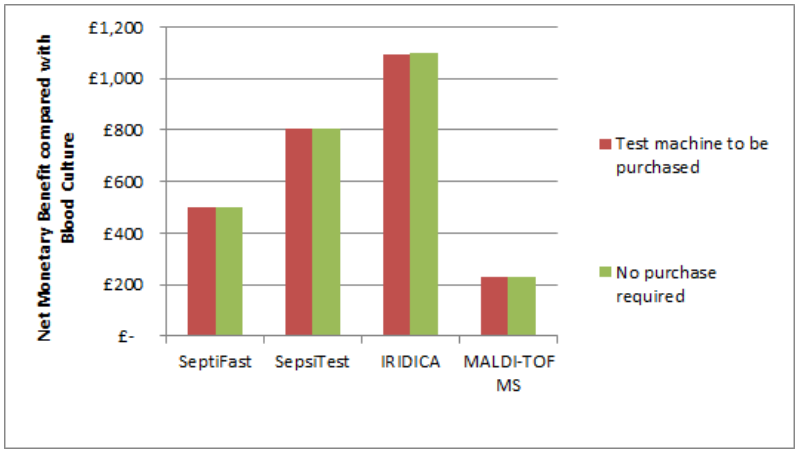


Figure 23: NMB assuming a MAICER of £30,000, a mortality rate of 29% and 68 blood samples to be analysed per day



3.5.2.1 Results from Base case 2 using the average clinician values and assuming a 30-day mortality rate of 13%

The estimated costs and estimated QALYs for each intervention and MALDI-TOF MS compared with blood culture when it is assumed that the machinery required for the intervention need to be purchased are shown in Table 39. The results where no additional machinery needs to be purchased are provided in Table 40.

It is seen that the cost per QALY values are relatively low for all interventions. It is assumed that any costs associated with allowing machines to be run on a seven days per week, 24 hours per day basis could be subsumed into the intervention costs whilst still producing ICERs that are below £20,000 per QALY gained. To illustrate this, if it was assumed that there were additional costs of £100,000 to operate SeptiFast continuously, then the ICER, assuming 17 samples to be analysed per day, and machine purchase, would be calculated as:

Incremental Cost: £201,782 (see Table 39) + £100,000 = £301,782
Incremental QALYs: 48.81(see Table 39)

ICER = £301,782 / £48.81 = £6183

Table 39: Estimated cost per QALY when it is assumed that only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 13%

	Incremental Cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year compared with blood culture†	Cost per QALY gained compared with blood culture
Assuming 2.4 blood samples per day			
SeptiFast	67,878	6.88	£9862
SepsiTest	-15,963	9.72	Dominating
IRIDICA	73,501	13.96	£5264
MALDI-TOF MS	-548	0.23	Dominating
Assuming 17 blood samples per day			
SeptiFast	201,782	48.81	£4134
SepsiTest	-343,990	68.96	Dominating
IRIDICA	-168,633	99.01	Dominating
MALDI-TOF MS	-13,094	1.65	Dominating
Assuming 68 blood samples per day			
SeptiFast	652,257	195.22	£3341
SepsiTest	-1,470,568	275.82	Dominating
IRIDICA	-674,533	396.06	Dominating
MALDI-TOF MS	-56,914	6.59	Dominating

Dominating means providing more QALYs for equal or lower cost
† These values include QALYs gained in subsequent years

Table 40: Estimated cost per QALY when it is assumed that no additional machinery needs to be purchased. Mortality rate assumed to be 13%

	Incremental Cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year compared with blood culture†	Cost per QALY gained compared with blood culture
Assuming 2.4 blood samples per day			
SeptiFast	64,107	6.88	£9314
SepsiTest	-22, 134	9.72	Dominating
IRIDICA	35,215	13.96	£2522
MALDI-TOF MS	-1322	0.23	Dominating
Assuming 17 blood samples per day			
SeptiFast	198,011	48.81	£4057
SepsiTest	-350,161	68.96	Dominating
IRIDICA	-206,919	99.01	Dominating
MALDI-TOF MS	-13,869	1.65	Dominating
Assuming 68 blood samples per day			
SeptiFast	637,173	195.22	£3264
SepsiTest	-1,476,739	275.82	Dominating
IRIDICA	-827,676	396.06	Dominating
MALDI-TOF MS	-57,688	6.59	Dominating

Dominating means providing more QALYs for equal or lower cost
† These values include QALYs gained in subsequent years

3.5.2.2 Results from Base case 2 using the average values from the clinician survey and assuming a 30-day mortality rate of 29%

The estimated costs and estimated QALYs for each intervention test compared with blood culture when it is assumed that the machinery required for the intervention need to be purchased are shown in Table 41. The results where no additional machinery needs to be purchased are provided in Table 42.

Table 41: Estimated cost per QALY when it is assumed that only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 29%

	Incremental Cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year compared with blood culture†	Cost per QALY gained compared with blood culture
Assuming 2.4 blood samples per day			
SeptiFast	67,878	15.35	£4421
SepsiTest	-15,963	21.69	Dominating
IRIDICA	73,501	31.15	£2360
MALDI-TOF MS	-548	0.52	Dominating
Assuming 17 blood samples per day			
SeptiFast	201,782	108.88	£1853
SepsiTest	-343,990	153.82	Dominating
IRIDICA	-168,427	220.88	Dominating
MALDI-TOF MS	-13,094	3.67	Dominating
Assuming 68 blood samples per day			
SeptiFast	652,257	435.50	£1498
SepsiTest	-1,470,568	615.30	Dominating
IRIDICA	-674,533	883.51	Dominating
MALDI-TOF MS	-56,914	14.69	Dominating

Dominating means providing more QALYS for equal or lower cost
† These values include QALYs gained in subsequent years

Table 42: Estimated cost per QALY when it is assumed that no additional machinery needs to be purchased. Mortality rate assumed to be 29%

	Incremental Cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year compared with blood culture†	Cost per QALY gained compared with blood culture
Assuming 2.4 blood samples per day			
SeptiFast	64,107	15.35	£4175
SepsiTest	-22,134	21.69	Dominating
IRIDICA	35,215	31.15	£1131
MALDI-TOF MS	-15,240	0.52	Dominating
Assuming 17 blood samples per day			
SeptiFast	198,011	108.88	£1819
SepsiTest	-350,161	153.82	Dominating
IRIDICA	-206,919	220.88	Dominating
MALDI-TOF MS	-159,840	3.67	Dominating
Assuming 68 blood samples per day			
SeptiFast	637,173	435.50	£1463
SepsiTest	-1,476,739	615.30	Dominating
IRIDICA	-827,676	883.51	Dominating
MALDI-TOF MS	-664,860	14.69	Dominating

Dominating means providing more QALYs for equal or lower cost
† These values include QALYs gained in subsequent years

3.5.2.3 Results from Base case 2 using the average values from the clinical survey, using the results from the data synthesis for SeptiFast

In the base case for SeptiFast it is assumed that data from Warhurst *et al.*[10] were the most appropriate as these were taken from an English population and this study was believed to be that with the best quality (see Table 6)

In an alternative scenario the impact of using the estimated results from the synthesis of diagnostic accuracy data (Figure 3) was explored. The midpoint values of 65% sensitivity and 86% specificity

were used, with the prevalence of sepsis identified by blood culture by episode assumed to be that reported in Table 6 of Warhurst *et al.*,[10] of 80/922 (8.7%). These data result in an estimated positivity rate of 18.4% for SeptiFast, in which 30.6% of the time the subsequent blood culture was also positive and 67% of the time the subsequent blood culture was negative. In this analysis a failure rate of 1.4% was assumed for SeptiFast based on a naïve pooling of failure results from Warhurst *et al.*[10] (69) and from Paolucci *et al.*[101] (100) divided by the number of samples analysed in the SeptiFast versus blood culture trials (11659).

The estimated cost per QALY values produced in the scenario where the results from the synthesis of diagnostic accuracy for SeptiFast are shown in Table 43 assuming a 13% mortality rate and in Table 44 assuming a 29% mortality rate.

It is seen that using the pooled results for SeptiFast rather than the Warhurst *et al.*[10] study would be more favourable to SeptiFast, with estimated although the ICERs are below £10,000 in all scenarios analysed regardless of the diagnostic accuracy data used.

Table 43: The estimated cost per QALY values of SeptiFast using the results from the synthesis of diagnostic accuracy data and assuming a mortality rate of 13%

	Incremental Cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year compared with blood culture†	Cost per QALY gained compared with blood culture
Assuming 2.4 blood samples per day			
Assuming SeptiFast machine purchase required	39,631	8.64	£4588
Assuming no purchase required	35,860	8.64	£4152
Assuming 17 blood samples per day			
Assuming SeptiFast machine purchase required	1477	61.25	£24
Assuming no purchase required	-2294	61.25	Dominating
Assuming 68 blood samples per day			
Assuming SeptiFast machine purchase required	-148,963	245.00	Dominating
Assuming no purchase required	-164,047	245.00	Dominating

Dominating means providing more QALYs for equal or lower cost
† These values include discounted QALYs gained in subsequent years

Table 44: The estimated cost per QALY values of SeptiFast using the results from the synthesis of diagnostic accuracy data and assuming a mortality rate of 29%

	Incremental Cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year compared with blood culture†	Cost per QALY gained compared with blood culture
Assuming 2.4 blood samples per day			
Assuming SeptiFast machine purchase required	39,631	19.27	£2057
Assuming no purchase required	35,860	19.27	£1861
Assuming 17 blood samples per day			
Assuming SeptiFast machine purchase required	1477	136.63	£11
Assuming no purchase required	-2294	136.63	Dominating
Assuming 68 blood samples per day			
Assuming SeptiFast machine purchase required	-148,963	546.53	Dominating
Assuming no purchase required	-164,047	546.53	Dominating

† These values include discounted QALYs gained in subsequent years

3.5.2.4 Results from Base case 2 using the individual clinician values

Cost effectiveness results produced by individual clinicians are provided in: Table 45 for SeptiFast; Table 46 for SepsiTest; Table 47 for IRIDICA; and Table 48 for MALDI-TOF MS. For conciseness only the cost per QALY values are presented with incremental costs and incremental QALY values omitted. Only the results assuming 2.4 tests a day have been documented as the purpose was to show the concordance between the individual clinicians, which is largely unaffected by the numbers of blood samples assumed to be analysed per day.

For all tests the answers were highly discordant between clinicians with answers ranging from dominated (higher cost with equal or less QALYs) to dominating (lower cost with equal or greater QALYs) indicating high levels of uncertainty in the assumed effectiveness of the interventions and MALDI-TOF MS.

Table 45: The estimated cost per QALY values for SeptiFast by individual clinicians using data from Warhurst *et al*.[10]

	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 13%	No additional machinery need to be purchased. Mortality rate assumed to be 13%	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 29%	No additional machinery need to be purchased. Mortality rate assumed to be 29%
Clinician 1	£6036	£5720	£2706	£2564
Clinician 2	£73,884	£71,870	£33,120	£32,217
Clinician 3	£2,137,101	£2,075,380	£958,011	£930,343
Clinician 4	Dominating	Dominating	Dominating	Dominating
Clinician 5	£42,886	£39,800	£19,225	£17,841
Clinician 6	Dominated	Dominated	Dominated	Dominated
Clinician 7	£2985	£2851	£1338	£1278

Dominating means providing more or equal QALYs at a reduced cost
Dominating means providing less or equal QALYs at an increased cost

Table 46: The estimated cost per QALY values for SepsiTest by individual clinicians

	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 13%	No additional machinery need to be purchased. Mortality rate assumed to be 13%	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 29%	No additional machinery need to be purchased. Mortality rate assumed to be 29%
Clinician 1	Dominating	Dominating	Dominating	Dominating
Clinician 2	£37,700	£34,874	£16,900	£15,633
Clinician 3	£1,294,903	£1,179,881	£580,474	£528,912
Clinician 4	Dominating	Dominating	Dominating	Dominating
Clinician 5	Dominating	Dominating	Dominating	Dominating
Clinician 6	Dominated	Dominated	Dominated	Dominated
Clinician 7	Dominating	Dominating	Dominating	Dominating

Dominating means providing more or equal QALYs at a reduced cost
Dominating means providing less or equal QALYs at an increased cost

Table 47: The estimated cost per QALY values for IRIDICA by individual clinicians

	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 13%	No additional machinery need to be purchased. Mortality rate assumed to be 13%	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 29%	No additional machinery need to be purchased. Mortality rate assumed to be 29%
Clinician 1	£6737	£4541	£3020	£2036
Clinician 2	£78,491	£64,489	£35,186	£28,909
Clinician 3	£2,288,469	£1,857,349	£1,025,865	£832,605
Clinician 4	Dominating	Dominating	Dominating	Dominating
Clinician 5	£50,386	£28,830	£22,587	£12,924
Clinician 6	Dominated	Dominated	Dominated	Dominated
Clinician 7	Dominating	Dominating	Dominating	Dominating

Dominating means providing more or equal QALYs at a reduced cost
Dominating means providing less or equal QALYs at an increased cost

Table 48: The estimated cost per QALY values for MALDI-TOF MS by individual clinicians

	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 13%	No additional machinery need to be purchased. Mortality rate assumed to be 13%	Only the machinery required for the intervention need to be purchased. Mortality rate assumed to be 29%	No additional machinery need to be purchased. Mortality rate assumed to be 29%
Clinician 1	£10,265	£255	£4601	£114
Clinician 2	Dominating	Dominating	Dominating	Dominating
Clinician 3	Did not answer this question			
Clinician 4	Dominated	Dominated	Dominated	Dominated
Clinician 5	Dominating	Dominating	Dominating	Dominating
Clinician 6	Dominated	Dominated	Dominated	Dominated
Clinician 7	Dominating	Dominating	Dominating	Dominating

Dominating means providing more or equal QALYs at a reduced cost
Dominating means providing less or equal QALYs at an increased cost

3.5.3 Results from the threshold analyses

Threshold analyses have been presented for each intervention in comparison with blood culture and with MALDI-TOF MS. It is assumed that where a comparison with MALDI-TOF MS is made that the unit already had a MALDI-TOF MS machine in place. The analyses have been undertaken assuming that an intervention needs to be purchased – it is assumed that if a laboratory already had one of the interventions in place that this would be routinely used.

Thresholds are reported for net reduction in mortality and net reduction in ICU length of stay combined, and for net reduction in antimicrobial costs and net reduction in ICU length of stay combined. All threshold results are presented per 100 positive tests and for 100 tests irrespective of the test result. For the net reduction in antimicrobial costs and net reduction in ICU length of stay combined there is no separate curve based on the MAICER as it is assumed that both factors affect cost only and the decision regarding cost effectiveness reduces to one of cost-minimisation.

Due to the number of figures presented the threshold analyses are contained in Appendix 8. Note in all analyses the diagnostic accuracy for SeptiFast has been taken from Warhurst *et al.*[10] as this was assumed a more representative study of English practice and was graded as higher study quality than the remaining studies (see Table 6).

In summary, relatively small mortality gains would be required for the interventions to achieve a cost per QALY gained of £20,000. The threshold levels compared with blood culture assuming that 2.4 samples per day need analysing are shown in Table 49. The values assuming that the comparator is MALDI-TOF MS is shown in Table 50.

These values assume no change in either of the two remaining parameters. All other scenarios require lower threshold values to attain a cost per QALY of £20,000 per QALY gained.

Table 49: Threshold levels require to achieve a cost per QALY gained of £20,000 assuming 2.4 samples needing analysing per day and a comparator of blood culture

	Per 100 tests			Per 100 positive tests		
	Reduction in 30-day mortality (lives)	Reduction in ICU length of stay (days)	Reduction in antimicrobial treatment costs (£)	Reduction in 30-day mortality (lives)	Reduction in ICU length of stay (days)	Reduction in antimicrobial treatment costs (£)
SeptiFast	0.09	19.45	205.54	0.62	133.82	1414.50
SepsiTest	0.07	14.15	149.53	0.39	83.46	882.15
IRIDICA	0.14	29.76	314.61	0.65	140.23	1482.28

Table 50: Threshold levels require to achieve a cost per QALY gained of £20,000 assuming 2.4 samples needing analysing per day and a comparator of MALDI-TOF MS

	Per 100 tests			Per 100 positive tests		
	Reduction in 30-day mortality (lives)	Reduction in ICU length of stay (days)	Reduction in antimicrobial treatment costs (£)	Reduction in 30-day mortality (lives)	Reduction in ICU length of stay (days)	Reduction in antimicrobial treatment costs (£)
SeptiFast	0.09	18.50	195.57	0.59	127.33	1345.90
SepsiTest	0.06	13.20	139.56	0.36	77.89	823.34
IRIDICA	0.13	28.82	304.65	0.63	135.79	1435.32

3.5.4 Results from the studies comparing SeptiFast with MALDI-TOF MS and comparing SepsiTest with MALDI-TOF MS

Two studies had a comparator of MALDI-TOF MS in addition to blood culture. These were Tafelski *et al.*,[114] where the index test was SeptiFast and Loonen *et al.*, [116] where the index text was SepsiTest. The cost effectiveness of these studies were estimated to see if these were concordant with the results produced by the indirect comparisons of SeptiFast and MALDI-TOF MS, and of SepsiTest and MALDI-TOF MS generated through the evidence provided by the clinical experts relative to blood culture.

The benefits associated with the interventions were amended to account for any benefit associated with a positive MALDI-TOF MS. Thus, for example, if SeptiFast had an estimated reduction in ICU stay of 0.607 per positive test and MALDI-TOF MS had a reduction of 0.175 day per positive test, then assuming that MALDI-TOF MS had a sensitivity of 0.798 compared with blood culture, which

was the reliable identification value at species level from Morgenthaler and Kostrzewa[30] the benefit of a positive SeptiFast test when the accompanying blood culture was positive in terms of ICU length of stay reduction would be calculated as:

0.607 - 0.175 x 0.798 which equals 0.468 days.

The costs of MALDI-TOF MS were subtracted from the costs of the tests based on the estimated number of tests performed. Results are provided in Table 51, assuming a mortality rate of 13% and Table 52 assuming a mortality rate of 29%. Given the relatively low ICERs for conciseness evaluations where machinery was not required to be purchased in relation to SeptiFast or SepsiTest were not reported. As no failures were mentioned within Tafelski *et al.*[114] or Loonen *et al.*,[116] it was assumed that there were none for the analyses presented.

The results indicate that SeptiFast appears more cost effective than MALDI-TOF MS when aggregated clinicians values are used. The results for SepsiTest are less conclusive with estimated values greater than £20,000 per QALY gained when a mortality rate of 13% is assumed, and with values below this when a mortality rate of 29% is assumed.

These results differ from those of the main analyses where SepsiTest appeared considerably more cost-effective than both MALDI-TOF MS and SeptiFast (refer to Figures 12 to 23).

Table 51: The estimated ICERs based on trials directly comparing interventions with MALDI-TOF MS assuming a mortality rate of 13% and that machinery needs to be purchased

	Incremental Cost per annum compared with MALDI-TOF MS (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year MALDI-TOF MS †	Cost per QALY gained MALDI-TOF MS
Assuming 2.4 blood samples per day			
SeptiFast	-38,345	12.40	Dominating
SepsiTest	84,087	2.41	£34,848
Assuming 17 blood samples per day			
SeptiFast	-499,681	87.95	Dominating
SepsiTest	417,246	17.11	£24,385
Assuming 68 blood samples per day			
SeptiFast	-2,128,094	351.79	Dominating
SepsiTest	1,599,875	68.44	£23,375

Dominating means providing more QALYs for equal or lower cost
† These values include discounted QALYs gained in subsequent years

Table 52: The estimated ICERs based on trials directly comparing interventions with MALDI-TOF MS assuming a mortality rate of 29% and that machinery needs to be purchased

	Incremental Cost per annum (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year†	Cost per QALY gained
Assuming 2.4 blood samples per day			
SeptiFast	-38,345	37.67	Dominating
SepsiTest	84,087	5.38	£15,621
Assuming 17 blood samples per day			
SeptiFast	-499,681	196.19	Dominating
SepsiTest	417,246	38.17	£10,931
Assuming 68 blood samples per day			
SeptiFast	-2,128,094	784.76	Dominating
SepsiTest	1,599,875	152.68	£10,479

Dominating means providing more QALYS for equal or lower cost
† These values include discounted QALYs gained in subsequent years

3.5.5 Results from studies comparing SeptiFast and SepsiTest simultaneously with blood culture

Two studies evaluated both SeptiFast and SepsiTest against blood culture. These were Schreiber *et al.*,[118] and Leitner *et al.*[117] The cost effectiveness of these studies were estimated to see if these were concordant with the results produced by the indirect comparisons of SeptiFast and SepsiTest generated through the evidence provided by the clinical experts relative to blood culture. Neither study mentioned the SeptiFast failure rate and thus it was assumed there were none. Within the comparison it was assumed that machinery would need to be purchased for both tests. The ICERs for SeptiFast compared with SepsiTest have been provided in Table 53 where the mortality rate was assumed to be 13% and in Table 54 when the mortality rate was assumed to be 29%. It is seen that in all of the scenarios that the ICER for SeptiFast compared with SepsiTest is greater than £30,000 per QALY gained. This conclusion is concordant with those of the main analyses as shown in Figures 12 to 23.

Table 53: The estimated ICERs based on trials directly comparing both SeptiFast and SepsiTest with blood culture assuming a mortality rate of 13% and that machinery needs to be purchased

Study		Incremental Cost per annum (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year†	Cost per QALY gained	Cost per QALY of SeptiFast compared with SepsiTest
	Assuming 2.4 blood samples per day				
Schreiber *et al.*[118]	SeptiFast	87,022	5.81	£14,975	£90,855
	SepsiTest	49,147	5.39	£9111	
Leitner *et al.*[117]	SeptiFast	67,686	6.95	£9739	Dominated
	SepsiTest	-7,910	9.27	Dominating	
	Assuming 17 blood samples per day				
Schreiber *et al.*[118]	SeptiFast	337,533	41.21	£8191	£74,363
	SepsiTest	117,708	38.25	£3077	
Leitner *et al.*[117]	SeptiFast	200,421	49.28	£4067	Dominated
	SepsiTest	-286,890	65.73	Dominating	
	Assuming 68 blood samples per day				
Schreiber *et al.*[118]	SeptiFast	1,195,262	164.83	£7252	£69,267
	SepsiTest	376,224	153.00	£2459	
Leitner *et al.*[117]	SeptiFast	646,815	197.13	£3,281	Dominated
	SepsiTest	-1,242,168	262.91	Dominating	

Dominating means providing more QALYS for equal or lower cost
Dominated means providing less QALYs at equal or a higher cost
† These values include QALYs gained in subsequent years

Table 54: The estimated ICERs based on trials directly comparing both SeptiFast and SepsiTest with blood culture assuming a mortality rate of 29% and that machinery needs to be purchased

Study		Incremental Cost per annum (£)	Incremental QALYs gained through number of avoided 30-day mortalities within one year†	Cost per QALY gained	Cost per QALY of SeptiFast compared with SepsiTest
	Assuming 2.4 blood samples per day				
Schreiber *et al.*[118]	SeptiFast	87,022	12.96	£6713	£40,728
	SepsiTest	49,147	12.03	£4084	
Leitner *et al.*[117]	SeptiFast	67,686	15.50	£4366	Dominated
	SepsiTest	-7,910	20.68	Dominating	
	Assuming 17 blood samples per day				
Schreiber *et al.*[118]	SeptiFast	337,533	91.92	£3672	£33,335
	SepsiTest	117,708	85.33	£1379	
Leitner *et al.*[117]	SeptiFast	200,421	109.94	£1823	Dominated
	SepsiTest	-286,890	146.62	Dominating	
	Assuming 68 blood samples per day				
Schreiber *et al.*[118]	SeptiFast	1,195,262	367.69	£3251	£31,051
	SepsiTest	376,224	341.31	£1102	
Leitner *et al.*[117]	SeptiFast	646,815	439.75	£1471	Dominated
	SepsiTest	-1,242,168	586.50	Dominating	

Dominating means providing more QALYS for equal or lower cost
Dominated means providing less QALYs at equal or a higher cost
† These values include QALYs gained in subsequent years

3.6 Interpretation of the independent economic model results

Forming conclusions based on the economic modelling is very difficult due to the lack of high-quality evidence regarding the impact of the interventions on hard patient outcomes such as sepsis-related mortality, length of stay in the ICU and on changes in the costs of antimicrobial therapy.

In Base case 1 where only data from the published literature on patient-related outcomes have been included all interventions were estimated to be dominated as there was no evidence that any

knowledge gained translated into a benefit for the patient and all interventions were associated with additional cost.

In Base case 2 parameter values were populated from estimates provided by clinical experts. Contrasting results were produced to those in Base Case 1. It was estimated that the cost per QALY for all interventions were below a threshold of £20,000 per QALY when using the average values provided by the clinical experts in all scenarios. However, when the results were broken down by individual clinician estimates it was seen that there was a wide variation in the cost per QALY values with greater than £20,000 per QALY estimates, when assuming 13% mortality rates and 2.4 samples analysed per day, for 4 out of 7 clinicians for SeptiFast, 2 out of 7 clinicians for SepsiTest, 4 out of 7 clinicians for IRIDICA and 2 out of 6 clinicians for MALDI-TOF MS. The clinical experts commented on the difficulty of the task of populating the model parameters and thus all results should be treated with caution.

The External Assessment Group also caution against forming conclusions from directly comparing the interventions although for completeness these results have been presented and a description of the key assumptions and data driving these comparative results is provided. IRIDICA is estimated to have much better sensitivity than either SeptiFast or SepsiTest, and this will be associated with an increase in QALYs and in cost reductions from ICU and hospital lengths of stay and in changes in antimicrobial costs. The interventions have similar specificity values, although that associated with IRIDICA is marginally lower than for SeptiFast or SepsiTest, this will also provide QALY gains and cost savings as the clinical experts believed false positives to be associated with an imperfect reference standard rather than an inaccurate test. Additionally the data provided by the expert clinicians indicated that a positive IRIDICA test would be more beneficial than the remaining tests. The QALY gains and cost savings associated with IRIDICA were sufficient to offset the more expensive costs associated with IRIDICA and to provide IRIDICA with the highest NMB. SepsiTest was seen to typically have the second highest NMB. This was not due to the inherent accuracy of the test, which has lower sensitivity than SeptiFast, but because of assumed lower cost per test and also a better estimated benefit per test provided by the clinicians. MALDI-TOF MS was presumed to be better than blood culture, but as this was conducted on much fewer blood samples, only those that are blood culture positive, the NMB was smaller than for any of the interventions.

Additional analyses undertaken using the results from multi-test studies that compared SeptiFast, SepsiTest and blood culture, when the data provided by clinicians were used, were concordant with Base case 2 in that SeptiFast has an estimated cost per QALY gained value of greater than £20,000 compared with SepsiTest. However, the indirect results produced when using studies directly

comparing to MALDI-TOF MS produced contrary results with SeptiFast estimated to dominate SepsiTest.

Given the discordant results between Base case 1 and Base Case 2 the External Assessment Group cannot confidently suggest any cost per QALY gained value for the interventions. It is clear that the majority of clinicians questioned believe the interventions are likely to be cost effective yet there are no conclusive data to show that the tests provide a benefit to patients.

Threshold analyses were produced as these may be helpful to decision makers in formulating guidance. It was seen that relatively small mortality gains would be required for the interventions to achieve a cost per QALY gained of £20,000 compared with standard practice.

The External Assessment Group comment that studies comparing the use of an intervention with standard practice, where the results from the tests are fed into a treatment management plan, are urgently needed to produce more definitive estimates of the cost per QALY gained. The RAPIDO study[31] is undertaking this for MALDI-TOF MS in addition to blood culture and clinical judgement. Whilst this study had recently completed, data analysis had not been fully conducted at the time of writing. When the results of the clinical and cost effectiveness of the addition of MALDI-TOF MS are known the best choice for standard practice in any future trial should be more certain.

4. DISCUSSION

4.1 Statement of principal findings

4.1.1 Clinical effectiveness

A comprehensive systematic review and meta-analysis (where applicable) was undertaken to evaluate the clinical effectiveness of three interventions (the LightCycler SeptiFast Test MGRADE; SepsiTest; and IRIDICA BAC BSI) in conjunction with clinical assessment for rapidly identifying bloodstream bacteria and fungi in people with suspected sepsis.

For the review of diagnostic test accuracy, 62 studies of varying methodological quality were included. Most of these studies were considered to be at risk of bias and having concerns regarding applicability. Pooled effects for sensitivity and specificity across 54 studies (comprising 10,010 patients) comparing SeptiFast with blood culture found that SeptiFast had a higher specificity (0.86, 95% CrI: 0.84 to 0.89) than sensitivity (0.65, 95% CrI: 0.60 to 0.71). Similarly, a higher specificity (0.74, 95% CI: 0.64 to 0.85) was observed than sensitivity (0.58, 95% CI: 0.30 to 0.86) in one study that compared SeptiFast with blood culture plus MALDI-TOF MS. However, due to the deficiencies in study quality in the included studies, these data may not be reliable and should be treated with caution. Moreover, the prediction intervals of the pooled estimates indicate a substantial amount of heterogeneity between studies, particularly for sensitivity. Reasons for the observed heterogeneity in sensitivity and specificity between studies were explored using meta-regression for several potentially relevant characteristics: age category (adults and children and neonates), antibiotic use at the time of blood sampling, community or health acquired infection, inclusion/exclusion of contaminants and patients with febrile neutropenia. There was no evidence to suggest that the pooled sensitivity and specificity was affected by these subgroups.

Pooled effects for sensitivity and specificity across four studies (comprising 460 patients) comparing SepsiTest with blood culture suggest that SepsiTest has a higher specificity (0.86, 95% CrI: 0.78 to 0.92) than sensitivity (0.48, 95% CrI: 0.21 to 0.74). Although, the pooled estimate indicates low sensitivity, the associated credible interval is large. Comparison with blood culture plus MALDI-TOF MS in a single study also showed higher specificities than sensitivity (0.96, 95% CrI: 0.92 to 1.00 and 0.11, 95% CrI: 0.00 to 0.23, respectively). Despite substantial amounts of heterogeneity between studies, analyses for potential causes of this heterogeneity could not be explored due to the small number of studies included. Due to the deficiencies in study quality in the included studies, the sensitivity and specificity data for SepsiTest may not be reliable and should be treated with caution.

The pooled effects for sensitivity and specificity across four studies (comprising 860 patients across two studies [data not reported for other studies]) comparing IRIDICA with blood culture suggest that IRIDICA has a higher specificity (0.84, 95% CrI: 0.71 to 0.92) than sensitivity (0.81, 95% CrI: 0.69

to 0.90), though the difference between sensitivity and specificity is small. Despite substantial amounts of heterogeneity between studies, analyses for potential causes of this heterogeneity could not be explored due to the small number of studies included. Due to the deficiencies in study quality in the included studies, the sensitivity and specificity data for IRIDICA may not be reliable and should be treated with caution.

Although 41 studies, across the three interventions, reported data on one or more intermediate (such as: time to pathogen identification; time to treatment; test failure rates; duration of stay in hospital or critical care units; and change in antimicrobial treatment plan) and/or clinical outcome measures (such as mortality), the majority of studies reported data for the whole patient cohort, as opposed to comparative data for the index and reference test. Few clinical trials have been conducted on the likely impact and safety of acting on the results of the real-time PCR assays in patients in any setting although three RCTs, all of SeptiFast, were identified. One did not investigate patient outcomes,[114] one was predominantly in febrile neutropenia patients and reported no significant difference in mortality,[112] length of stay (ICU or hospital) or fever duration. The third RCT was published in abstract form only and did not report a difference in mortality.[102]

Given the potentially fatal consequences of removing treatment from patients with sepsis it is not anticipated that negative tests in isolation would be acted upon in clinical practice were an intervention introduced. In addition, the three interventions provide very limited data regarding antimicrobial sensitivity. Definitive data on this is needed to be determined, if possible, via standard culture methods undertaken in parallel with the interventions.

4.1.2 Cost effectiveness

A systematic review of the literature was undertaken to identify cost effectiveness analyses relating to the interventions. Two of these were within-study analyses, one using propensity scoring to match patients,[105] and one not.[91] The remaining two presented results from modelling studies, with one evaluating SeptiFast[68] and one evaluating an IRIDICA-PLEX-ID hybrid.[130] The External Assessment Group noted limitations with all four studies and constructed a *de novo* mathematical model and reported results under a number of scenarios. In Base Case 1, only documented statistically significant benefits associated with the tests were included, resulting in an estimation that all of the interventions provided no benefit. In order to investigate alternative scenarios, clinicians from the Diagnostic Appraisal Committee and who are authors of this report were asked, for each intervention, to provide estimates of the benefits associated with a positive test: this formed Base case 2. At the aggregate level all of the interventions were estimated to have cost per QALY gained values below £20,000. However, these results must be taken with caution as the clinicians noted the difficulty of the task and there was a wide divergence of opinion amongst the individual clinicians.

Additional analyses, using the data provided by clinicians, were undertaken to assess whether the estimated results were altered by analysing individual studies that assessed an intervention versus MALDI-TOF MS, or where two interventions were compared simultaneously within a study. The results from the studies against MALDI-TOF MS were concordant with those produced in Base case 2 for individual interventions; however, indirectly SeptiFast appeared to dominate SepsiTest, which did not occur in Base case 2. For trials that assessed SeptiFast and SepsiTest simultaneously the ICER for SeptiFast compared with SepsiTest was consistently greater than £30,000 per QALY which was concordant with Base case 2. It is commented that the results of the evaluation of SeptiFast with SepsiTest are driven by the relative costs of each test rather than the diagnostic accuracy and also the assumed benefits assigned to each test by the expert clinicians. The External Assessment Group notes that the specificities of the tests are comparable but the sensitivity of SeptiFast is estimated to be greater than that for SepsiTest.

To provide potentially useful information to the Diagnostic Appraisal Committee threshold analyses were undertaken in relation to 30-day mortalities prevented, reduction of number of days in the ICU and in terms of reduced antimicrobial costs.

4.2 Strengths and limitations of the assessment

4.2.1 Clinical effectiveness

The strengths of this systematic review are that it was conducted using robust methods including the development of a pre-specified protocol, comprehensive searching of published and unpublished evidence (including contact with clinical experts in the field and checking evidence submitted by the companies that manufacture the tests), study selection (including adjudication by three independent clinical experts), and data extraction by a minimum of two independent reviewers and a formal assessment of methodological quality. Statistical evaluation of diagnostic test accuracy was undertaken using statistically rigorous methods, allowing for the correlation between sensitivity and specificity, and potential between study heterogeneity. Reasons for the heterogeneity in sensitivity and specificity between studies were explored using meta-regression and parameter estimates were produced using Markov Chain Monte Carlo simulation.

The assessment of methodological quality was generally hampered by poor quality of reporting in the included SeptiFast, SepsiTest and IRIDICA studies, with the majority of studies being classified at unclear risk of bias on most assessment domains. Although a number of abstracts were included in the current systematic review, differences often occur between data reported in conference abstracts and their corresponding full reports; however, differences in results are usually not very large.[41]

The pooled estimates of sensitivity and specificity for SeptiFast, SepsiTest and IRIDICA were estimated assuming that the reference standard was 100% sensitive and specific; however, this is unlikely to be the case. In practice, a wide range of factors are known to influence the diagnostic accuracy of blood cultures. For example this may include antimicrobial treatment prior to blood sampling, low blood sample volumes, lack of replicate blood culture sets, delays in incubation and contamination during sampling. (Public Health England, 2014a[12] and Warhurst *et al.* 2015)[10] As a result, the reported estimates of sensitivity and specificity are likely to be biased (underestimated) compared to those that would be obtained using a perfect reference standard. In addition, diagnostic metrics in the included studies were measured using different units: patients, sample episodes or species/pathogen level. Such analyses create a 'unit of analyses' error and may have contributed to the heterogeneity in the results.

Although no other systematic reviews or meta-analysis were identified for SepsiTest or IRIDICA, the present overall findings and conclusion for SeptiFast compared with blood culture were consistent with the review and meta-analysis by Dark *et al.* (2014)[50] with pooled effects for sensitivity and specificity (across 41 SeptiFast studies, which were also included in the current review) of 0.68 (95% CI: 0.63 to 0.73) and 0.86 (95% CI: 0.84 to 0.89, respectively. An earlier systematic review of SeptiFast by Chang *et al.* (2013)[154]) observed similar specificities (0.92, 95% CI: 0.90 to 0.95) but higher sensitivities (0.75, 95% CI: 0.65 to 0.83) across 34 SeptiFast studies. This review included a number of studies that were excluded in the present review due to publication type (foreign language, n=2) or not meeting our inclusion criterion of 'suspected sepsis' (n=2). In addition, Chang *et al.* (2013)[154] pooled studies comparing SeptiFast results against various reference standards to produce composite overall diagnostic accuracy metrics. These factors may have contributed to the higher diagnostic performance metrics than that found in the present review and Dark *et al.* (2014).[50]

4.2.2 Cost effectiveness

A systematic review of the cost effectiveness literature associated with the interventions was undertaken. The External Assessment Group noted limitations with the identified evidence and therefore constructed a *de novo* model. A strength of the modelling work undertaken is that a framework for modelling interventions which provide rapid information on blood stream bacteria and fungi has been established. The framework allows for there to be a benefit associated with false positive tests and thus explicitly incorporates the fact that blood culture, with or without MALDI-TOF MS, is an imperfect reference test.

A fundamental limitation is that there are little robust data to populate the mathematical model. The External Assessment Group attempted to reduce this limitation by asking clinical experts to provide data to be used in an evaluation. The robustness of any conclusions are severely limited given the

feedback from clinicians regarding the difficulty of the task and also due to the large heterogeneity of results produced from individual clinicians which range from the interventions dominating to the interventions being dominated.

Further limitations are acknowledged in the model, which was simplistic, although none are expected to influence the conclusion that until further research is performed that no robust assessment of cost effectiveness can be made. The limitations of the model include: the lack of modelling regarding: antimicrobial stewardship benefits; the cost implications of any service reconfiguration required to move to a 24 hour a day, 7 day a week service; any training costs required; any utility differential in survivors with and without any intervention; the possibility that only a sequencer need be purchased to run SepsiTest; that the estimates for the sensitivity of MALDI-TOF MS have been used at species level; and that any discounts associated with undertaking large quantities of tests have been omitted.

4.3 Uncertainties

4.3.1 Clinical effectiveness

All of the included studies compared the index test with a reference standard (blood culture with or without MALDI-TOF MS). No studies were identified that compared all of the index tests of interest directly with each other (end to end studies). In addition, there are very limited, robust data at present that report the impact of interventions on hard clinical outcomes such as mortality and reduced length of stay in critical care or in hospital.

4.3.2 Cost effectiveness

The key uncertainty relates to the estimated cost effectiveness of each intervention. The results produced by the External Assessment Group indicate that at an aggregate level clinicians believe the interventions to provide information that if acted upon would improve key patient outcomes of mortality or ICU length of stay. However, there are no data currently available to support these views and no definitive conclusions can be provided until further research is undertaken.

5. CONCLUSION

5.1 Implications for service provision

Given the considerable uncertainty in the cost effectiveness results produced for each intervention it is uncertain what the implications within the NHS would entail. Were the interventions deemed to be a cost effective use of resources then it is likely that reconfigurations of working practice would be required in order that the interventions could provide results more quickly than under the present system.

5.2 Research Recommendations

Despite the growing evidence base for all three interventions, a number of key issues need to be addressed. First, all future clinical studies incorporating SeptiFast, SepsiTest and IRIDICA need to be well-designed and reported in accordance to the Standards for the Reporting of Diagnostic accuracy studies statement.[155] Once robust diagnostic accuracy data has been established there is a need for a pragmatic trial where the results from the interventions are allowed to change patient management and for these results to be compared to standard practice in order to allow robust estimates of the clinical and cost effectiveness of an intervention to be estimated. A process evaluation, running alongside such a pragmatic trial, may also be of value to understand how the tests are used in the NHS. At present there are very limited data that report the impact of interventions on hard clinical outcomes such as mortality and reduced length of stay in critical care units. Any such trials should wait until the results from the RAPIDO trial[31] are published in order that key information on the clinical utility of MALDI-TOF MS compared with blood culture is known. Finally, research into logistical issues such as the numbers of hospitals serviced by the machine and the number of days that the machine operates to determine the optimal use of the interventions in England is required.

5.3 Conclusions

5.3.1 Clinical effectiveness

SeptiFast, SepsiTest and IRIDICA appear to have higher specificity values than sensitivity values. However, due to the deficiencies in study quality in the included studies, these data may not be reliable and should be treated with caution. Moreover, there are no head to head comparisons of all these tests and there are limited, robust data that report the impact of interventions on hard clinical outcomes such as mortality and reduced length of stay in critical care units. The data that do exist have not shown any intervention to produce a statistically significant improvement. In order to produce a definitive conclusion on the clinical effectiveness of the interventions appropriate studies need to be conducted (see 7.4).

5.3.2 Cost effectiveness

There is considerable uncertainty associated with all analyses within this assessment and a definitive estimate of the cost effectiveness of each intervention cannot be provided. This is largely due to the limitations of the evidence base. The studies recommended in Section 5.4 would reduce this uncertainty. Threshold analyses have been provided that may allow decision makers to estimate whether the interventions are likely, or not, to meet a level at which the decision makers would consider the interventions to be cost effective.

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7. APPENDICES

Appendix 1: Literature search strategies for the review of clinical effectiveness – A MEDLINE example

Database searched:	Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R)
Platform or provider used:	Ovid SP
Date of coverage:	1948 to May 2015
Search undertaken:	Initial search February 2015
Updated search:	May 2015

1. exp Sepsis/
2. sepsis.mp.
3. septic?emia.mp.
4. Shock, Septic/
5. ((septic or endotoxic or toxic) adj shock).tw.
6. Bacteremia/
7. bacter?emia.mp.
8. Fungemia/
9. fung?emia.mp.
10. Systemic Inflammatory Response Syndrome/
11. sirs.mp.
12. blood$ infection$.tw.
13. blood poison$.tw.
14. or/1-13
15. septifast.mp.
16. lightcycler.mp.
17. 15 or 16
18. 14 and 17
19. sepsitest.mp.
20. iridica.mp.
21. (plex id or plex-id).mp.
22. or/19-21
23. exp Polymerase Chain Reaction/
24. polymerase chain reaction$.tw.
25. pcr$.mp.
26. Gene Amplification/
27. Nucleic Acid Amplification Techniques/
28. or/23-27
29. Genes, Bacterial/ or Genes, Fungal/
30. (exp bacteria/ or exp Fungi/) and exp Nucleic Acids/
31. ((bacteri$ or fung$) adj3 (dna or gene$ or nucleic acid$)).tw.
32. blood culture$.tw.
33. or/29-32
34. 14 and 28 and 33
35. 18 or 22 or 34
36. Animals/ not (Humans/ and Animals/)
37. 35 not 36
38. limit 37 to yr="2006 -Current"

Appendix 2: The QUADAS-2 tool (adapted) for the methodological assessment of diagnostic studies[51]

Quality domain	Scoring	Summary judgement
RISK OF BIAS		
Patient selection: Was a consecutive or random sample of patients enrolled? Was a case-control design avoided? Did the study avoid inappropriate exclusions?	'Yes' if states consecutive or random 'No' if states another method of patient sampling/selection 'Unclear' if unclear or not reported 'Yes' 'No' 'Unclear' if insufficient information provided 'Yes' if the study provides explicit exclusion criteria and appropriately select participants that are typical of patients with blood stream infection/suspected sepsis 'No' if the study has made inappropriate exclusions from the group it set out to select i.e., unrepresentative of people with blood stream infection/suspected sepsis. 'Unclear' if insufficient information provided	Could the selection of patients have introduced bias? 'Low risk' if all domains are Yes 'High risk' if one or more domain is No 'Unclear risk' anything in between
Index test: Were the index test results interpreted without knowledge of the results of the reference standard?	'Yes' if index test was interpreted without knowledge (blind) of the results of the reference standard or the index test was clearly interpreted before the reference standard was known. 'No' if results of reference standard were already known 'Unclear' if insufficient details are provided	Could the conduct or interpretation of the index test have introduced bias? 'Low risk' if all domains are Yes 'High risk' if one or more domain is No 'Unclear risk' anything in between
Reference standard: Is the reference standard likely to correctly classify the target condition? Were the reference standard results interpreted without knowledge of the results of the index test?	'Yes' if clinical standard described and is consistent with published standard operating procedures. 'No' if reference standard falls short of standard operating procedures. 'Unclear' if insufficient information provided. 'Yes' if the reference standard was interpreted blind to the index test or the reference standard was clearly interpreted before the index test was known. 'No' if the results of the index test were known. 'Unclear' if insufficient information is provided	Could the conduct or interpretation of the reference standard have introduced bias? 'Low risk' if all domains are Yes 'High risk' if one or more domain is No 'Unclear risk' anything in between

Quality domain	Scoring	Summary judgement
Flow and timing: Was there an appropriate interval between index test(s) and reference standard?	'Yes' if reference standard and index tests performed on blood samples drawn at the same time 'No' if reference standard and index tests not performed on blood samples drawn at different times 'Unclear' if insufficient information is provided	
Did all patients receive a reference standard?	'Yes' if all participants who received the index test also verified using the reference test 'No' if not all (or some) of the participants who received the index test also underwent the reference test (partially verified). If all participants did not receive the reference test, how many did not (of the total) 'Unclear' if insufficient information is provided	Could the patient flow have introduced bias? 'Low risk' if all domains are Yes 'High risk' if one or more domain is No 'Unclear risk' anything in between
Did patients receive the same reference standard?	'Yes' if the same reference test was used regardless of the index test results 'No' if different reference tests are used depending on results of the index tests. If different reference tests are used, what were the reasons and how many participants were involved 'Unclear' if insufficient information is provided	
Were all patients included in the analysis?	'Yes' if all patients who were recruited/enrolled into the study were included in the analysis or if sufficient explanation is provided for any discrepancy 'No' if there are participants excluded from the analysis and no/insufficient explanation is given for any discrepancy 'Unclear' if insufficient information is given to assess whether any patients were excluded from the analysis.	
APPLICABILITY		
Patients: Are there concerns that the included patients and settings do not match the review question?	Scored in relation to the description of included patients	'Yes' if the sample is unrepresentative of people with blood stream infection/suspected sepsis 'No' if characteristics of participants are well described and typical of patients with blood stream infection/suspected sepsis 'Unclear' if characteristics are not well described

Quality domain	Scoring	Summary judgement
Index test: Is there concern that the index test, its conduct, or interpretation, differ from the review question, i.e., CE protocol followed?	Scored in relation to the CE mark protocol for SeptiFast, SepsiTest and IRIDICA	'Yes' if CE mark protocol for SeptiFast, SepsiTest and IRIDICA is not followed 'No' if CE mark protocol for SeptiFast, SepsiTest and IRIDICA is followed 'Unclear' if insufficient details provided
Reference standard: Is there concern that the target condition as defined by the reference standard does not match the review question?	Scored in relation to description of the reference standard	'Yes' if full details of reference standard are not provided e.g., the reference standard may be free of bias, but the target condition that it defines may differ from the target condition specified in the review question. 'No' if full details are provided 'Unclear' if insufficient details provided

Appendix 3: Clinical effectiveness review - table of excluded studies with rationale

Table 55: Studies excluded from the clinical review

	Author, year	Reason for exclusion
1.	Anon (2010)[1]	Trial record with no study results
2.	Anon (2011)[2]	Protocol: Septifast EVAMICA trial
3.	Anon (2012)[3]	Trial record of Idelevich *et al.* (2011)[4]
4.	Anon (2013)[5]	Replaced (protocol) by full text paper reported by Rodrigues *et al.* (2013)[6]
5.	Anon (2014)[7]	Ongoing Septifast trial - estimated completion January 2017
6.	Abbott Molecular Inc. (2014)[7]	Replaced (package insert) by full text paper reported by Metzgar *et al.* (unpublished)[8]
7.	Afsharpaiman *et al.* (2007)[9]	Cultured samples (positive)
8.	Al-Zahrani *et al.* (2015)[10]	Not intervention (test) of interest
9.	Arabestani *et al.* (2014)[11]	Cultured samples (positive)
10.	Avolio *et al.* (2010)[12]	Diagnostic metrics data included in Avolio *et al.* (2014)[13] (confirmed by authors)
11.	Avolio *et al.* (2010)[14]	Replaced (abstract) by full text paper reported by Avolio *et al.* (2010)[12]
12.	Baraki *et al.* (2012)[15]	Specimens from tissue samples only
13.	Bauer *et al.* (2010)[16]	Cultured samples (positive)
14.	Bernaschi *et al.* (2010)[17]	Replaced (abstract) by full text paper reported by Lucignano *et al.* (2011)[18]
15.	Bilkovski *et al.* (2015)[19]	Replaced (abstract/poster) by full text paper reported by Vincent *et al.* (accepted, in press)[20]
16.	Bingold *et al.* (2009)[21]	Not target population (patients scheduled for orthoptic liver transplant)
17.	Brearley *et al.* (2014[22]	Replaced (abstract/poster) by full text paper reported by Vincent et al (accepted, in press)[20]
18.	Burdino *et al.* (2012)[23]	Replaced (abstract) by full text paper reported by Burdino *et al.* (2014)[24]
19.	Cambau *et al.* (2013)[25]	Insufficient information to allow calculation of diagnostic 2x2 table (Septifast EVAMICA trial)
20.	Casalta *et al.* (2009)[26]	Not target population (patients with infective endocarditis)
21.	Chaidaroglou *et al.* (2010)[27]	Not target population (implantable ventricular assist device patients suspected of infection)

22.	Chaidaroglou *et al.* (2011)[28]	Not target population (thoracic allograft recipients)
23.	Chaidaroglou *et al.* (2012)[29]	Not target population (implantable ventricular assist device patients suspected of infection)
24.	Chaidaroglou *et al.* (2012)[30]	Not target population (thoracic allograft recipients)
25.	Chaidaroglou *et al.* (2013)[31]	Not target population (thoracic allograft recipients)
26.	Chan *et al.* (2009)[32]	Cultured samples (positive/ negative)
27.	Clerici *et al.* (2009)[33]	Insufficient information to allow calculation of diagnostic 2x2 table
28.	Conen *et al.* (2009[34]	Not target population (patients with suspected native or prosthetic valve infective endocarditis)
29.	Dark *et al.* (2011)[35]	Replaced (protocol) by full text paper reported by Warhurst *et al.* (2015)[36]
30.	Diamante *et al.* (2010)[37]	Foreign Language (Italian)
31.	Disque *et al.* (2008)[38]	No outcome data
32.	Disque *et al.* (2010)[39]	Foreign Language (German)
33.	Disque *et al.* (2010)[40]	Replaced (abstract) by full text paper reported by Wellinghausen *et al*. (2009)[41]
34.	Disque *et al.* (2010)[41]	Replaced (abstract) by full text paper reported by Wellinghausen *et al.* (2009)[42]
35.	Disque *et al.* (2011)[43]	No comparator (study investigating the influence of blood volume)
36.	Disque *et al.* (2012)[44]	Specimens (liquid and tissue) from different body sites and mixed population)
37.	Disque *et al.* (2012)[45]	Specimens (liquid and tissue) from different body sites and mixed population)
38.	Draz *et al.* (2013)[46]	Not intervention (test) of interest
39.	Dubska *et al.* (2012)[47]	Not target population (patients with solid malignancy)
40.	Elwan *et al.* (2009)[48]	Not intervention (test) of interest
41.	Enomoto *et al.* (2009)[49]	Not intervention (test) of interest
42.	Gosiewski *et al.* (2014)[50]	Not intervention (test) of interest
43.	Greco *et al.* (2012)[51]	Replaced (abstract) by full text paper reported by Barbanti *et al.* (2015)[52]
44.	Greco *et al.* (2014)[53]	Insufficient information to allow calculation of diagnostic 2x2 table
45.	Grif *et al.* (2012)[54]	Specimens (liquid and tissue) from different body sites
46.	Haag *et al.* (2013)[55]	Specimens (liquid and tissue) from different body sites

47.	Halasz *et al.* (2012)[56]	Insufficient information to allow calculation of diagnostic 2x2 table (not a diagnostic study)
48.	Halliday *et al.* (2014)[57]	Letter/comment with no details of intervention
49.	Hettwer *et al.* (2009)[58]	Insufficient information to allow calculation of diagnostic 2x2 table
50.	Holmes *et al.* (2014)[59]	Not target population (haematological malignancies)
51.	Horvath *et al.* (2013)[60]	Not intervention (test) of interest
52.	Idelevich *et al.* (2011)[4]	Replaced (abstract) by full text paper reported by Idelevich *et al.* (2015)[61]
53.	Irwin *et al.* (2012)[62]	Not target population (patients with increased levels of C-reactive protein)
54.	Jordan *et al.* (2006)[63]	Not intervention (test) of interest
55.	Jordana-Lluch *et al.* (2013)[64]	Not intervention (test) of interest (used older extraction method on PLEX-ID system, thus not comparable to the current IRIDICA platform)
56.	Josefson *et al.* (2010)[65]	Replaced (abstract) by full text paper reported by Josefson *et al*. (2011)[66]
57.	Kalenka *et al.* (2009)[67]	Not target population (patients with suspected ventilator associated pneumonia)
58.	Kalenka *et al.* (2009)[68]	No comparator (i.e. not versus blood culture)
59.	Kaleta *et al.* (2011)[69]	Cultured samples (positive)
60.	Karam *et al.* (2012)[70]	Not intervention (test) of interest
61.	Kim *et al.* (2011)[71]	Foreign Language - (Korean)
62.	Kuhn *et al.* (2011)[72]	Replaced (abstract) by full text paper reported by Kuhn *et al.* (2011)[73]
63.	Kuhn *et al.* (2011)[73]	Not target population (patients with suspected infectious endocarditis and used valvular and blood samples for analysis)
64.	Lefort *et al.* (2012)[74]	Not target population (patients with Candida endocarditis)
65.	Lehmann *et al.* (2009)[75]	Not intervention (test) of interest
66.	Leli *et al*. (2014)[76]	Study aim to develop prediction model from positive SepsitFast results only.
67.	Liberto *et al*. (2006)[77]	Not intervention (test) of interest
68.	Liu *et al*. (2014)[78]	Not intervention (test) of interest
69.	Lodes *et al.* (2011)[79]	Foreign language (German)

70.	Markota *et al.* (2013)[80]	Diagnostics metrics data (from patients and samples) included in a full text study by Markota *et al*. (2014)[81]
71.	Martinez *et al.* (2010)[82]	Coagulase-negative staphylococci (CoNS) detection only
72.	Mencacci *et al.* (2012)[83]	Not target population (patients with suspected infective endocarditis)
73.	Mencacci *et al.* (2012)[84]	Insufficient information to allow calculation of diagnostic 2x2 table
74.	Merisescu *et al.* (2012)[85]	Cultured samples (blood and/or fluids)
75.	Merisescu *et al.* (2014)[86]	Klebsiella pneumonia detection only (no details on sample type, comparator methods or useable outcome data)
76.	Meyer *et al.* (2014)[87]	Specimens from CSF samples
77.	Molina *et al*. (2008)[88]	Foreign Language (Spanish)
78.	Mongelli *et al*. (2015)[89]	Not target population (febrile patients with suspected bacteraemia)
79.	Moore *et al.* (2014)[90]	Not intervention (test) of interest
80.	Mundy and Hiller (2010)[91]	Review
81.	Niederbracht *et al.* (2013)[92]	Coagulase-negative staphylococci (CoNS) detection only
82.	Nieman *et al.* (2010)[93]	Preclinical validation study.
83.	Nieman *et al.* (2011)[94]	Insufficient information to allow calculation of diagnostic 2x2 table
84.	Novak-Frazer *et al.* (2012)[95]	Specimens from whole blood and wound swabs (and insufficient information to allow calculation of diagnostic 2x2 table)
85.	Ohlin *et al*. (2012)[96]	Not intervention (test) of interest
86.	Orszag *et al.* (2013)[97]	Not target population (patients supported by extracorporeal membrane oxygenation)
87.	Ortiz *et al.* (2012)[98]	Insufficient information to allow calculation of diagnostic 2x2 table
88.	Palomares *et al.* (2009)[99]	Replaced (abstract) by full text paper reported by Palomares *et al*. (2009)[100]
89.	Pleskova *et al.* (20110[101]	Cultured samples (positive) and unclear if cancer patients have sepsis
90.	Popov *et al.* (2011)[102]	Foreign Language (Russian)
91.	Raineri *et al.* (2009)[103]	Insufficient information to allow calculation of diagnostic 2x2 table
92.	Ratanarat *et al*. (2007)[104]	Not intervention (test) of interest

93.	Reier-Nilsen *et al.* (2009)[105]	Not intervention (test) of interest
94.	Rogina *et al.* (2014)[106]	Insufficient information to allow calculation of diagnostic 2x2 table
95.	Sahre *et al.* (2007)[107]	Foreign Language (German)
96.	Sakka *et al.* (2010)[108]	Replaced (abstract) by full text paper reported by Wellinghausen *et al.* (2009)[42]
97.	Sampath (2011)[109]	Conference abstract not available
98.	Santolaya *et al.* (2011)[110]	Not intervention (test) of interest
99.	Schaub *et al.* (2009)[111]	Diagnostic metrics data included in Schaub *et al*. (2014)[112]
100.	Shaat *et al*. (2013)[113]	Not intervention (test) of interest
101.	Sitnik *et al.* (2011)[114]	Replaced (abstract) by full text paper reported by Sitnik *et al*. (2015)[115]
102.	Skvarc *et al.* (2012)[116]	Insufficient information to allow calculation of diagnostic 2x2 table
103.	Steinmann *et al.* (2011)[117]	Replaced (abstract) by full text paper reported by Rath *et al*. (2012)[118]
104.	Stubljar *et al.* (2013)[119]	Not intervention (test) of interest
105.	Tafelski *et al.* (2013)[120]	Replaced (abstract) by full text paper reported by Tafelski *et al*. (2015)[121]
106.	Torres-Martos *et al*. (2013)[122]	Foreign Language (Spanish)
107.	Tsalik *et al.* (2009)[123]	Insufficient information to allow calculation of diagnostic 2x2 table
108.	Vrsajkov *et al.* (2014)[124]	Not intervention (test) of interest
109.	Warhurst *et al.* (2015)[125]	Replaced (Health Technology Assessment monograph) by full text journal paper reported by Warhurst *et al*. (2015)[36]
110.	Zerweck *et al.* (2010)[126]	Not target condition (patients undergoing induction therapy and or stem cell transplantation)
111.	Ziegler *et al*. (2014)[127]	Diagnostic metrics data (secondary analysis) included in Josefson *et al*. (2011)[66]

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2. Anon. Diagnosis of Septicaemia by Detection of Microbial DNA in Blood in Severe Infections. *Clinicaltrials Gov* 2011.http://ClinicalTrials.gov/show/NCT00709358

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Appendix 4: Study and population characteristics of the included studies

Table 56: Study characteristics

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
SINGLE INDEX TEST STUDIES - SEPTIFAST					
Dierkes *et al.* (2009)[66] Germany Sponsor/funding NR However SeptiFast provided free of charge by Roche Full-text	Single gate Retrospective study Sampling method NR 8 months July 2006-March 2007)	Surgical and medical wards	Intensive/critical care	Inclusion criteria NR: chart review	NR
Raglio *et al.* (2006)[60] European multicentre Sponsor/funding NR, Abstract	Single gate Study type NR Sampling method NR Sampling period NR	NR	NR	Reported that patients fulfilling SIRS criteria were patients included	NR
Bingold *et al.* (2007)[61] Germany Sponsor/funding NR Abstract	Single gate Study type NR Sampling method NR Sampling period NR	Anaesthesiological/ surgical ICU	Intensive/critical care	Reported that significant elevated inflammatory parameters (PCT, IL-6, LBP) at study entry; informed consent were patients included	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Klemm *et al.* (2007)[62] Germany Sponsor/funding NR Abstract	Single gate Study type NR Sampling method NR Sampling period NR	Intensive care	Intensive/critical care	Reported that clinical suspicion for sepsis (fever (> 38°C) or hypothermia (< 36°C) and additionally leucocytosis (> 12.000/µl), leucopenia (< 4.000/µl), leucocyte left shift (> 10%), tachycardia (> 90/min), tachypnoe (> 20/min) or hyperventilation (pCO2 < 33 mmHg)) were patients included	NR
Lodes *et al.* (2008)[63] Germany Sponsor/funding NR Abstract	Single gate Study type NR Consecutive sample Sampling period NR	Surgical intensive care unit	Intensive/critical care	Reported that surgical patients with SIRS on intensive care were patients included	NR
Louie *et al.* (2008)[46] USA Roche Diagnostics, and grant from Nat Inst of Biomedical Imaging and Bioengineering, NIH Full-text	Single gate Prospective study Sampling method NR 34 months	University Medical Centre	Emergency department, in hospital and intensive/critical care	Inclusion criteria - adults from emergency department, intensive care unit and general medicine with suspected bloodstream infection and at least two SIRS criteria	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Mancini *et al.* (2008)[45] Italy Roche Full-text	Single gate Study type NR Consecutive sample 2 months	Haematology Unit	In hospital and unclear if intensive/critical care	Reported that patients were seventy-three (70.9 %) samples were drawn from heavily neutropenic patients	NR
Vince *et al.* (2008)[64] Croatia, two centres Partly supported by Ministry of Science, Education and Sports of the Republic of Croatia Correspondence	Single gate Study type NR Sampling method NR Sampling period NR	Intensive care unit (n=17), outside intensive care unit (n=9) and Department of Haematology following bone marrow or peripheral blood stem cell transplantation (n=10)	In hospital and intensive/critical care	Reported that patients with a clinical diagnosis of sepsis who were treated with antimicrobial therapy were patients included	NR
Dark *et al.* (2009)[65] UK Sponsor/funding NR Correspondence	Single gate Study type NR Sampling method NR Sampling period NR	Samples from neuro-injured, general surgical and general internal medicine patients	Intensive/critical care	Reported that patients with new episodes of suspected blood stream infection were patients included	NR
Gimeno *et al*. (2009)[67] Spain, single centre NR Abstract	Single gate Prospective Sampling method NR	NR	NR	Haemotological patients with febrile neutropenia	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
	5 months (dates NR)				
Lehmann *et al.* (2009)[68] Germany, 5 centres Supported, in part, by Roche Diagnostics Full-text	Single gate Retrospective study Sampling consecutive Sampling period NR	ICU, emergency room, medical and surgical wards	Emergency department, in hospital and intensive/critical care	Inclusion criteria were ≥18 yrs of age, suspected sepsis, a blood culture drawn, and subsequent antibiotic treatment initiation or change	NR
Lodes *et al.* (2009)[69] Germany, Sponsor/funding NR Full-text	Single gate Prospective study Consecutive sample 4 months (May to August 2006)	Surgical intensive care unit	Intensive/critical care	Surgical patients with SIRS and subsequent need of intensive care	NR
Palomares *et al.* (2009)[70] Spain, Single centre Sponsor/funding NR Abstract	Single gate Study type NR Sampling method NR Sampling period NR	Intensive care unit	Intensive/critical care	Reported that patients with suspected BSI and 2 SIRS were patients included	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Paolucci *et al.* (2009)[71] Italy Sponsor/funding NR Correspondence	Single gate Retrospective study Sampling method NR Sampling period NR	NR	NR	Reported that patients were newborns aged older than three days with late onset sepsis	NR
Varani *et al.* (2009)[72] Italy, three centres Sponsor/funding NR Full-text	Single gate Study type NR Sampling method NR Sampling period NR	Paediatric Oncology and Haematology Unit,	In hospital and unclear if intensive/critical care	Reported that immunocompromised patients with haematological malignancies in whom sepsis was suspected were patients included	NR
von Lilienfeld-Toal. *et al.*(2009)[73] Germany, single centre Roche Molecular Diagnostics (All reagents, instruments, and disposables were obtained from Roche Molecular Diagnostics) Full-text	Single gate Prospective Sampling method NR 16 months (Sept 2001 to Feb 2002; Apr 2003 to Jan 2004)	Tertiary care hospital Haemotology ward	In hospital	NR - included patients with febrile neutropenia after chemotherapy for hematological malignancies.	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Westh *et al.* (2009)[74] Germany, Multicentre (n=6) Research funding from Roche Diagnostics Full-text	Single gate Study type NR Sampling method NR 5 months (June to October 2004)	NR	NR	Specific inclusion criteria NR - all patients included were clinically suspected to have bacterial or fungal sepsis	NR
Berger *et al.* (2010)[75] Austria Sponsor/funding NR Abstract	Single gate Study type NR Sampling method NR Sampling period NR	Neonatology	Neonatal unit	Very low birth weight infants	NR
Bloos *et al.* (2010)[76] Germany, France Roche Full-text	Single gate Prospective study Sampling method NR Dec 2005- April 2007	Intensive care	Intensive/critical care	Inclusion criteria - presence of severe sepsis or septic shock according to the ACCP/SCCM consensus criteria	Exclusion criteria - < 18 years of age or previous enrolment in this trial
Lamoth *et al.* (2010)[77] Switzerland, single centre Roche Diagnostics Full-text	Single gate Prospective Consecutive 14 months (Sept 2006 to Nov 2007)	Univesity Hospital Isolation ward	In hospital	NR - included febrile neutropenic adult hematological patients undergoing induction or consolidation chemotherapy for acute leukemia or autologous hematopoietic stem cell transplantation	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Lehmann *et al.* (2010)[78] Germany, 2 centres Research funding, reagents, and equipment from Roche Diagnostics Full-text	Single gate Prospective study Sampling method NR Sampling period NR	Surgical intensive care units	Intensive/critical care	Reported that all adult patients who were clinically suspected of suffering from severe sepsis of bacterial or fungal origin. Inclusion followed after independent decision of the physician in charge to call for a blood culture were patients included	NR
Maubon *et al.* (2010)[79] France, single centre Roche Full-text	Single gate Prospective study Consecutive sample 12 months	Teaching Hospital	In hospital and unclear if intensive/critical care	Reported that patients with solid or haematological malignancies were admitted for suspected infection with at least one sign of sepsis, with or without organ dysfunction Reported that	NR
Reguerio *et al.* (2010)[80] Spain, single centre Partly supported by the Spanish Ministerio de Ciencia e Innovacion Full-text	Single gate Study type NR Sampling method NR 13 months, May 2007 to May 2008	Intensive Care and Anaesthology Services	In hospital and intensive/critical care	Reported that patients who all met criteria for SIRS and suspected sepsis on admission were patients included	NR

Author (year), Country, Source of funding, Manuscript type	**Study Design, Study type, Sampling method, Sampling period**	**Department and wards providing care for acutely unwell patients or critical care units**	**Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist**	**Inclusion criteria (illness aetiology)**	**Exclusion criteria**
Soki *et al*. (2010)[81] Hungary, Single centre Sponsor/funding NR Abstract	Single gate Study type NR Sampling method NR Sampling period NR	Intensive care unit and haematology department	In hospital and intensive/critical care	Reported that patients displaying symptoms of sepsis with or without antibiotic therapy were patients included	NR
Tsalik *et al.* (2010)[82] USA, two centres NIH grant and Roche Full-text	Single gate Study type NR Sampling method NR 66 months	University Medical Centre ED (Trauma Centre) and Veterans Affairs Medical Centre	Emergency department	Inclusion criteria - patients were considered for inclusion in the study if they had a known or suspected infection on the basis of clinical data at the time of screening and if they exhibited two or more signs of the systemic inflammatory response syndrome (SIRS) within a 24-h period	Exclusion criteria- patients were excluded if they were 18 years old, if they had an imminently terminal comorbid condition, or if they were participating in an on-going clinical trial. Only subjects admitted to the hospital and for whom blood culture results were available were patients included in this analysis
Wallet *et al.* (2010)[83] France, single centre Sponsor/funding NR Roche Molecular Diagnostics provided materials to perform the study. Full-text	Single gate Consecutive study Sampling method NR 6 months	ICU	Intensive/critical care	All patients with fever (≥38^{o} C) or hypothermia (≤36o C) were eligible	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Yanagihara *et al.* (2010)[84] Japan, Multicentre (n=3) Research funding, reagents, and equipment from Roche Diagnostics Full-text	Single gate Prospective study Sampling method NR 1 year (May 2007 to April 2008)	Departments of surgery, haematology, emergency, cardiopulmonary and ICU	In hospital and Emergency department	Reported that patients (treated or untreated) with SIRS caused by bacterial or fungal infection, and for whom blood culture was considered to be required for identification of the causative pathogens were patients included	NR
Bravo *et al.* (2011)[85] Spain Sponsor/funding NR Full-text	Single gate Study type NR Non-consecutive sample 8 months (Feb - Sept 2009)	Medical and Surgical ICU	In hospital and intensive/critical care	Inclusion criteria - Development of a febrile episode in neutropenic or ICU patients that required hospital admission or occurred during hospital stay	Exclusion criteria - receipt of empirical antibiotic treatment prior to blood sampling for analysis
Hettwer *et al.* (2011)[86] Germany Roche Full-text	Single gate Prospective study Sampling method NR Aug 2006-March 2009	Emergency dept.	Emergency department	Reported that patients > 18 years and admitted with clinical signs prompting physician to draw blood culture were patients included	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Josefson *et al.* (2011)[87] Sweden, Single centre Research grant: County Council of Örebro. Reagents and technical assistance: Roche Diagnostics. Full-text	Single gate Prospective study Consecutive sample 1 year (October 2007 to September 2008)	Department of infectious diseases	In hospital	Reported that all patients who were subjected to blood culture at the department and gave their informed consent were patients with HIV and with hepatitis B and C infections for local laboratory safety reasons.	NR
Lucignano *et al.* (2011)[88] Italy, single centre Sponsor/funding NR Full-text	Single gate Retrospective study Sampling method NR 26 months	Intensive care units and surgery; oncology, haematology and neonatology; emergency department and paediatrics	In hospital and intensive/critical care	Reported that patients with clinical suggestion of systemic inflammatory response syndrome (SIRS) with suspected bacterial or fungal infection, availability of a filled-out questionnaire with demographic, clinical, and laboratory information , and collection of paired blood samples for SeptiFast and two blood samples for cultures from a peripheral vein or a central venous line Reported that	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Obara *et al.* (2011)[89] Japan Partly funded by Roche Full-text	Single gate Study type NR Consecutive sample 6 months (September 2004 to March 2005)	University Hospital	Emergency department, in hospital and intensive/critical care	Reported that patients with suspected bacterial/fungal infection and at least two criteria of the systemic inflammatory response syndrome Reported that	NR
Vrioni *et al.* (2011)[90] Greece, two centres Sponsor/funding NR Abstract	Single gate Study type NR Sampling method NR Sampling period NR	NR	NR	Reported that patients with presumed sepsis in intensive care unit were patients included	NR
Alvarez *et al.* (2012)[91] Spain Sponsor NR Full-text	Single gate Retrospective study Sampling method NR (Cost-minimisation study) 12 Months	Intensive care unit	Intensive care unit	Patients with a diagnosis of severe sepsis or septic shock	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Grif *et al.* (2012)[92] Austria Pfizer Full-text	Single gate Prospective study Consecutive sample 3 months Jan 2009- Mar 2009	ICU and general wards	In hospital and intensive/critical care	Reported that patients with presumed sepsis (>2 SIRS criteria) were patients included	NR
Guido *et al.* (2012)[93] Italy Sponsor/funding NR Full-text	Single gate Study type NR Consecutive sample 12 months Jan 2010- Dec 2010	Haematology dept.	In hospital and unclear if intensive/critical care	Reported that patients with febrile neutropenia (temperature > 38.0 C) and neutrophil count <0.5x10 L in presence of acute and/or chronic blood disorders or bone marrow transplant were patients included	NR
Lodes *et al.* (2012)[94] Germany, single centre Sponsor/funding NR Full-text	Single gate Study type NR Consecutive sample 20 months	ICU Department of Surgery	Intensive/critical care	Reported that patients were patients in ICU with clinical diagnosis of sepsis within last 24h at risk of abdominal sepsis were patients with non-threatening SIRS	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Mauro *et al.* (2012)[95] Italy, single centre Sponsor/funding NR Full-text	Single gate Study type NR Sampling method NR Sampling period NR	Department of Paediatric Oncology and Department of Internal Medicine	In hospital and unclear if intensive/critical care	Reported that immunocompromised patients, which was defined as patients with any of the following: neutropenia (neutrophil count b1 × 103/μL), exposure to immunosuppressive agents, haematological malignancy, or solid tumour Reported that	NR
Pasquilani *et al.* (2012)[96] Italy, single centre Sponsor/funding NR Full-text	Single gate Study type NR Consecutive 5 months	Department of Internal Medicine	In hospital and unclear if intensive/critical care	Reported that patients with suspected of having systemic inflammatory response syndrome (SIRS) caused by bacterial or fungal infection and for whom blood culture was performed for causative pathogen identification Reported that.	NR
Rath *et al.* (2012)[97] Germany, single centre Sponsor/funding NR Full-text	Single gate Prospective study Sampling method NR 24 months, May 2009 to April 2011	Department of General, Visceral, and Transplant Surgery	Intensive/critical care	Reported that intensive care unit (ICU) patients with suspected sepsis according to the criteria of the ACCP/SCCM were patients included	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Tschiedel *et al.* (2012)[98] Germany, multicentre Sponsor/funding NR Full-text	Single gate Retrospective study Sampling method NR 19 months (May 2009 to Dec 2010)	Different hospitals	In hospital and intensive/critical care	Reported that critically ill patients with symptoms of systemic infection were patients included	NR
Herne *et al.* (2013)[99] Estonia Sponsor/funding NR Full-text	Single gate Retrospective study Sampling method NR Mar 2007- July 2011	Acute and intensive care,	In hospital and intensive/critical care	Reported that patients with severe infection from intensive care or other parts of hospital. Clinically proven sepsis or septic shock or severe infection without known etiologic agent were patients included Patients with only blood culture or SeptiFast collected samples	NR
Kasper *et al.* (2013)[100] Austria, Roche Molecular Diagnostics provided materials to perform the study. Full-text	Single gate Study type NR Sampling method NR Sampling period NR	NR	NR	Specific inclusion criteria NR - very low birth weight infants when infection was suspected after 72h life were patients included	NR
Paolucci *et al.* (2013)[101] Italy, single centre The University of	Single gate Prospective Consecutive 22 months (Jun	Haematology and the Paediatric Oncology and Haematology Unit,	In hospital	NR - included severely neutropaenic with haematological malignancies	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Bologna ; the Italian Ministry of Education, University, and Research MIUR Full-text	2008 to Mar 2010)	St. Orsola-Malpighi University Hospital, Bologna, Italy.			
Rodrigues *et al.* (2013)[102] Brazil Sponsor NR Abstract	Single gate Prospective RCT Sampling method NR Seven months	NR – patients from cardiology hospital	NR	Included adult patients over 18 years of age staying more than 48 hours in hospital, with clinical suspicion of sepsis	NR
Avolio *et al.* (2014)[103] Italy, Single centre Sponsor/funding NR Full-text	Single gate Prospective study Consecutive sample 3 years (September 2008 to December 2011)	Emergency and Intensive care unit	Emergency department and intensive/critical care	Inclusion criteria - Older than 18 years; admitted to the ED of the S. Maria degli Angeli Hospital (Pordenone, Italy) with suspected BSIs; and at least two criteria of the systemic inflammatory response syndrome	NR
Burdino *et al.* (2014)[104] Italy Sponsor/funding NR Full-text	Single gate Study type NR Sampling method NR Oct 2008- Dec 2012	Samples from infectious disease, ICU, cardio, internal and surgical departments	In hospital and intensive/critical care	Reported that patients had signs and symptoms of sepsis as defined by a systemic inflammatory response syndrome (SIRS) with suspected bacterial or fungal infections for each patient were patients included	

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Mancini *et al.* (2014)[105] Italy, 2 centres Roche Diagnostics Full-text	Single gate Retrospective data compared with prospective data Sampling method NR (propensity score study) Sampling period 34 months retrospective, 24 months prospective	Haematology and bone marrow transplant unit	In hospital	Haematological patients with suspected sepsis	NR
Markota *et al.* (2014)[106] Slovenia, single centre Sponsor/funding NR Full-text	Single gate Prospective study Sampling method NR 13 months, September 2011 to September 2012	Medical Intensive Care Unit (ICU)	Intensive/critical care	Specific inclusion criteria NR - adults who fulfilled the criteria for severe sepsis or septic shock were patients included	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Ozkaya-Parlakay *et al.* (2014)[107] Turkey, single centre Sponsor/funding NR Full-text	Single gate Prospective study Consecutive sample 30 months (Sept 2009 to Feb 2012)	Paediatrics dept. University Hospital	NR	Reported that patients were patients between 1 month and 17 years without immune deficiency were patients included	NR
Schaub *et al.* (2014)[108] Switzerland, single centre Research grants from the University Basel, the Department of Internal Medicine, and Roche Full-text	Single gate Prospective study Consecutive sample 20 months (June 2007 to Jan 2009)	University Hospital	Emergency department	Inclusion criteria - patients presenting to the ED with suspected sepsis Both patients with and without prior antimicrobial therapy were patients included.	Exclusion criteria were age <18 years
Sitnik *et al.* (2014)[109] Brazil, Multicentre (n=2) Roche Diagnostics donated all material multiplex polymerase chain reaction testing and training on test workflow Full-text	Single gate Prospective study Consecutive sample 11 months (December 2008 to October 2009)	Intensive care unit Emergency room and oncology patients	Intensive/critical care (and oncology patients)	Reported that all patients with infection plus two or more SIRS were patients included	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Barbanti *et al*.(2015)[122] Italy, single centre NR Abstract	Single gate NR Consecutive Months NR (2009 to 2013)	Haematology and bone marrow transplant unit	In hospital	Haemotological patients with febrile neutropenia	NR
Calitri *et al*. (2015)[111] Italy, single centre None Full-text	Single gate Retrospective Sampling method NR 37 months (Sep 1 2009 to Sep 30 2012)	Wards (various), Intensive care unit	In hospital and intensive/critical care	Paediatric patients with suspected sepsis, febrile neutropenia, fever without focus or localised infective focus	NR
Idelevich et al. (2015)[112] Germany, single centre Partly funded by Roche diagnostics and Pfizer Full-text	Single gate Prospective RCT Sampling method NR 28 months (May 2010 to Sept 2012)	University Hospital Münster	NR	Patients who developed febrile neutropenia according to IDSA criteria. Afebrile neutropenic patients fulfilling sepsis criteria were also eligible to participate. Patient inclusion took place from Sunday afternoon until noon on Friday.	Patients with of non-infectious causes of fever were excluded.
Tafelski *et al.* (2015)[114] Germany, Multicentre Roche Deutschland Full-text	RCT Randomised, double blind, parallel group trial Consecutive sample	Intensive care units	Intensive/critical care	Patients were eligible for study inclusion when they presented with signs of sepsis of suspected abdominal or pulmonary origin, caused by an unknown pathogen when blood culture diagnostics were indicated. Sepsis was	Exclusion criteria were age <18 years, pregnancy, police custody, missing or withdrawn informed consent or participation in another prospective clinical study.

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
	20 months (August 201 to March 2012)			defined as suspected or proven infection causing systemic inflammation with at least two of the following: (i) leucocyte count <4 or >12 /nl; (ii) body temperature <36 C or fever >38 C; (iii) tachypnoea >20/min or hyperventilation (paCO2 <32 mmHg); (iv) tachycardia >90 bpm.5 Infections were defined by the treating physicians using standardized criteria for infection management implemented on the participating wards since 2006. All patients were patients included only once even if they had multiple episodes of sepsis during their ICU stay. Additionally, infection onset was required to be <72 h, to reduce the risk of detecting persistent circulating DNA. Additional criteria (due to limited availability of trained staff to undertake PCR testing during evening and weekends) to ensure an adequate comparison, patients	

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
				were only enrolled in the study between 6pm and 6am because PCR test were only available for 12 h	
Warhurst *et al.* (2015)[113] UK, Multicentre (n=4) UK NIHR HTA programme Full-text	Single gate Prospective study Consecutive sample 2 years 6 months (30 July 2010 to 31 January 2013)	Critical care	Intensive/critical care	Inclusion criteria - patients (aged ≥16 years) in a critical care setting and identified by the treating clinician as having clinical suspicion of developing a suspected bloodstream infection after ≥48 h of hospital admission or recent exposure to hospital care. Suspicion of bloodstream infection was based on the development of two or more systemic inflammatory response syndrome (SIRS) criteria. Patient inclusion was then based entirely on the clinical decision to perform urgent blood culture investigations.	Exclusion criteria were: patients already recruited into the study, except where a subsequent, new episode of suspected healthcare-associated bloodstream infection had developed, and/or patients placed on an end-of-life care pathway.
SINGLE INDEX TEST STUDIES - SEPSITEST					

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Wellinghausen *et al*. (2009)[48] Germany, multicentre Bundesministerium ür Wirtschaft und Technologie Full-text	Single gate Prospective Sampling method NR Eleven months	Departments of Medicine, Paediatrics, and Surgical Intensive Care	In hospital and intensive/critical care	ICU patients with SIRS or sepsis, haematology/oncology patients with fever and neutropenia (one site), or patients with other forms of hereditary or acquired immunodeficiency and fever (one site)	NR
Loonen *et al*. (2014)[116] Netherlands, single centre Sponsor/funding NR Full-text	Single gate Retrospective study - data acquired retrospectively from the laboratory information system Sampling method NR 5 Months (Nov to Dec 2011 and Oct to Dec 2012)	Hospital Emergency Department	Emergency dept.	Reported that patients were patients with ≥ 2 SIRS criteria and clinical signs of infection presenting at the emergency department were patients included	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Nieman et al. (unpublished)[115] [redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
SINGLE INDEX TEST STUDIES - IRIDICA					
Bacconi *et al*. (2014)[49] USA, single centre NR but majority authors are employees of Ibis Biosciences (an Abbott company) Full-text	Single gate Prospective study Sampling method NR 4 months (Jan to Aril 2012)	Hospital Emergency Department	Emergency dept.	Inclusion criteria - subjects were considered eligible if they were ≥18 years old, were having blood cultures drawn as part of clinical care, and were able to provide informed consent.	NR
Delco-Volante *et al*. (2015)[120] Country NR Abbott Conference presentation	Single gate Prospective study Consecutive sample 17 months (August 2013 December 2014)	NR	NR	NR - patients included neonates (<28 days old) if the treating physicians diagnosed a suspected sepsis and intended to treat them with antibiotics	NR

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Vincent *et al*. (in press)[121] Belgium, UK, Switzerland, France, Poland, Germany [redacted] Full-text	[redacted]	ICU	Intensive/critical care	[redacted]	[redacted]
Metzgar *et al.* (unpublished)[119] [redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TWO INDEX TEST STUDIES – SEPTIFAST AND SEPSITEST					

Author (year), Country, Source of funding, Manuscript type	Study Design, Study type, Sampling method, Sampling period	Department and wards providing care for acutely unwell patients or critical care units	Clinical setting - Community, Emergency Department, in-hospital, intensive/critical care, general/specialist	Inclusion criteria (illness aetiology)	Exclusion criteria
Leitner *et al*. (2013)[117] Austria, Sponsor/funding - Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz Full-text	Single gate Study type NR Sampling method NR Sampling period NR	NR	NR	Reported that critically ill patients were patients included	NR
Schreiber *et al*. (2013)[118] Germany, single centre Molzym GmbH & Co. KG, Sirs-Lab GmbH and Roche Diagnostics GmbH provided materials Full-text	Single gate Prospective study Sampling method NR 4 months (April to July 2009)	Department of Intensive Care Medicine	Intensive/critical care	Inclusion criteria - minimum age of 18 years, as well as clinical symptoms and signs consistent with the diagnosis of sepsis according to the sepsis criteria of the German Sepsis Competence Network	NR

Table 57: Population characteristics

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
SINGLE INDEX TEST STUDIES - SEPTIFAST					
Dierkes *et al.* (2009)[66]	Adults, age 55 median 49/77 (64%) male Thirty-five of the patients (45%) were immunocompromised Concomitant antibiotic therapy at the time of specimen collection had been administered in 61 patients with 83 samples (81%) studied Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Patients with presumed sepsis - not defined	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 77 patients analysed 99 paired samples
Raglio *et al.* (2006)[60]	Adults/children/neonates NR, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 74 patients analysed 114 paired samples
Bingold *et al.* (2007)[61]	Not stated, age NR n male NR Adults/children NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	100% severe or septic shock	Severe sepsis and septic shock according to the S2 guidelines of the German Society of Sepsis	Site of infection NR Other symptoms NR	21 patients included n patients not followed up NR Samples from 21 patients analysed 134 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Klemm *et al.* (2007)[62]	Adults/children/neonates NR, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Patients enrolled in this study had evidence for a new focus of infection and a clinical suspicion for sepsis - not defined	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 44 patients analysed 56 paired samples
Lodes *et al.* (2008)[63]	Adults/children/neonates NR, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Sepsis was classified according to the ACCP/SCCM consensus conference criteria[2]	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 137 patients analysed 358 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Louie *et al.* (2008)[46]	Adults >18yrsMale, median 47 9range 18 to 80); female, median 46 (range 18 to 91) 122/200 (61%) male AIDS, immunosuppressant, 8 (4%) Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Septic shock/MODS, 2 (1%)	Suspected BSIs and at least two criteria of the systemic inflammatory response syndrome	Site of infection NR Respiratory/pneumonia, 44 (22%); Trauma/abscess, 34 (17%); Cancer/neutropenic fever, 29 (14.5%); Line infection, 23 (11.5%); Cellulitis, 17 (8.5%); Urinary/pyelonephritis, 14 (7%); Endocarditis/cardiovascular, 12 (6%); AIDS, immunosuppressant, 8 (4%); Gastrointestinal, 6 (3%); Postoperative fever, 5 (2.5%); Septic shock/MODS, 2 (1%); Other, 6 (3%)	200 patients included n patients not followed up NR Samples from 200 patients analysed 200 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Mancini *et al.* (2008)[45]	Adults (21 to 69), age 47 (range 21 to 69) 23/34 (67.6%) male n immunocompromised NR - all haematological malignancies Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Underlying disease, no. (%): AML, 14 (41.2%); ALL, 5 (14.7%); HD, 5 (14.7%); NHD 3, (8.8%); MDS, 2 (5.9%); CLL, 2 (5.9%); MM, 2 (5.9%); Biph. AL, 1 (2.9%)	34 patients included n patients not followed up NR Samples from 34 patients analysed 103 paired samples
Vince *et al.* (2008)[64]	Adults/children/neonates NR, age NR n male NR Haematology patients following bone marrow or peripheral blood stem cell transplantation (10/39, 25.6%) 36/36 (100%) patients (empirical antimicrobial therapy) Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 36 patients analysed 39 paired samples
Dark *et al.* (2009)[65]	Adults, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	50 patients included n patients not followed up NR Samples from 50 patients analysed 90 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Gimeno *et al.* (2009)[67]	Adults/children, NR Age NR n Male NR n immunocompromised NR Antibiotics prior to blood sample 19/19 (100%) All patients received antibiotics and/or antifungal prophylaxis Previous antibiotic exposure NR	NR	NR	NR	n patients recruited NR n patients not followed up NR n patients analysed 19 n paired blood samples 45
Lehmann *et al.* (2009)[68]	Adults, mean age 54.8 (range 18–92) 268/436 male n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	NR	Sepsis was defined according to the SCCM/ACCP consensus conference guidelines of 1992[4]	Intra-abdominal sepsis, 136 ; Nosocomial pneumonia, 112; Community-acquired pneumonia, 19; Multiorgan dysfunction syndrome, 13; Catheter-related sepsis, 61; Neutropenic fever, 47; Pyelonephritis, 24; Genitourinary infection, 13; Wound infection, 10; Bone/joint infection, 14; Other, 102 Other symptoms NR	n patients recruited NR 436 patients with467 episodes of antimicrobial treatment were included in the study in total Paired blood samples NR

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Lodes *et al.* (2009)[69]	Adults (range NR), age 60.5 (14.7) 30/52 (58%) male n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Sepsis was classified according to the ACCP/SCCM consensus conference criteria[2]	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 52 patients analysed 258 paired samples
Palomares *et al.* (2009)[70]	Adults, age NR n male NR n immunocompromised NR 68/73 patients (93%) receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 73 patients analysed 76 paired samples
Paolucci *et al.* (2009)[71]	Neonates aged 3 days and older, age NR (however, newborns were aged 3 days and older) n male NR One was affected by primary congenital immunodeficiency Antibiotics prior to blood sample NR (however, 3 blood culture-newborns received antibiotics prior to blood sampling) Previous antibiotic exposure NR	Clinical suspicion of sepsis	Sepsis was based on the presence of at least one clinical sign suggestive of clinical sepsis, and elevated C-reactive protein >2.0mg.	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 34 patients analysed n paired blood samples NR

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Varani *et al.* (2009)[72]	Adults and children, age NR - 85 adults and 15 children n male NR All immunocompromised All patients received antibiotics and antifungal prophylaxis Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR - patients with neutropenia or fever with signs and symptoms of infection	Site of infection NR 50 acute myeloid leukaemia, 17 acute lymphoblastic leukaemia, 15 lymphoma, 7 multiple myeloma, and 3 chronic myeloproliferative disorders), solid tumours (6 patients), or other diseases (1 case of autoimmune thrombocytopenia, 1 hemophagocytic lymphohistiocytosis)	100 patients included n patients not followed up NR Samples from 100 patients analysed 130 paired samples
von Lilienfeld-Toal. *et al.*(2009)[73]	Adults Median age 60 (IQR 49 to 66) 38/70 (54%) male n immunocompromised NR Antibiotics prior to blood sample None Previous antibiotic exposure NR	NR	NR	Acute myeloid leukaemia 50 (71%), Acute lymphatic leukaemia 8 (11%), Non-Hodgkin lymphoma 4 (6%), Multiple myeloma 2 (3%), Myelodysplastic syndrome 2 (3%), Aplastic anemia 2 (3%), Metastatic carcinoma 2 (3%)	n patients recruited NR n patients not followed up NR n patients analysed 70 (119 episodes) n paired blood samples 784

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Westh *et al.* (2009)[74]	Adults/children/neonates NR, age NR n male NR n immunocompromised NR Limited details provided regarding patients receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 359 patients analysed 558 paired samples
Berger *et al.* (2010)[75]	Neonates, age NR n male NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Clinical sepsis suspicion – sepsis not defined	Site of infection NR Other symptoms NR	38 patients included n patients not followed up NR Samples from 38 patients analysed 38 paired samples
Bloos *et al.* (2010)[76]	Adults, age 66 68.5% male None immunocompromised 95.8% on antibiotics (unclear if prior to blood sampling) Previous antibiotic exposure NR	100% severe sepsis or septic shock Severity of disease SOFA score 10 for entire sepsis cohort	Severe sepsis or septic shock according to ACCP/SCCM consensus criteria[2]	Lung (40%) Abdomen (16.9%) Blood stream (9.3%) Catheter-related (9.3%) Mechanical ventilation 81.7%	n patients recruited NR n patients not followed up NR Samples from 142 patients analysed 236 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Lamoth *et al.* (2010)[77]	Adults Median age 54 (range 17 to 71) 53/86 (62%) male n immunocompromised NR Antibiotics prior to blood sample 144 (61%) samples drawn under AB therapy Previous antibiotic exposure NR	NR	NR	Acute myeloid leukemia 37 (43), Acute lymphoblastic leukemia 9 (10), Lymphoma 12 (14), Multiple myeloma 22 (26), Other haematological malignancies 6 (7), Chemotherapy Induction/consolidation for acute leukemia 45 (52), Autologous stem cell tranplant 37 (43) Other chemotherapy 4 (5)	n patients recruited NR n patients not followed up NR n patients analysed 86 n paired blood samples 237

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Lehmann *et al.* (2010)[78]	Adults (range 18-84), age 58.37 72/108 (67%) male n immunocompromised NR - 18.5% (20/108) had neoplasms Antibiotics prior to blood sample NR Previous antibiotic exposure NR	100% severe sepsis	Severe sepsis was classified according to the ACCP/SCCM consensus conference criteria[2]	Abdominal sepsis (n=35 patients); sepsis following CV surgery (n=28); pneumonia/ARDS (n=23); tissue infection following trauma (n=15); osteomyelitis (n=2); genitourinary infection (n=2); mediastinitis (n=2); catheter related (n=1) Chronic co-morbidities (some had multiple): neoplasms (18.5%); liver failure (16.7%); NYHA III/IV heart failure (50.9%); chronic renal failure (n=45.4%); Diabetes (19.4%)	n patients recruited NR n patients not followed up NR Samples from 108 patients analysed 453 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Maubon *et al.* (2010)[79]	Adults/children/neonates NR, age Of the 110 patients: 56.3 (13.7) 57/110 (60.9%) male n immunocompromised NR - all haemoto -oncology study cohorts 97/110 (88.2%) receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	64/110 (58%) had severe sepsis and 27/110 (25%) had septic shock	Suspected infection with at least one sign of sepsis, with or without organ dysfunction, as defined by the SIRS criteria.	Site of infection NR Acute leukaemia, 48 (43.6%); Lymphoma, 28 (25.5%); Myeloma, 10 (9.1%); Other haematological malignancy, 9 (8.2%); Solid tumour 15 (13.6%)	110 patients included Reports on pathogens identified in 50 patients with documented sepsis not followed up Samples from 110 patients analysed 110 paired samples
Reguerio *et al.* (2010)[80]	Adults, 21 to 92, age 64 (range 21 to 92) 53/72 (73.6%) male n immunocompromised NR - 6/72 (8.3%) oncology Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease entire cohort APACHE II: 0-4, n=0; 5-9, n=2; 10-14, n=9; 15-19, n=15; 20-24, n=12; 25-29; n=15; 30-34, n=11; >34, n=8	NR	Site of infection NR Basal Disease: respiratory, 18; cardiovascular, 18; alcoholism, 10; oncologic, 6; digestive, 6; psychiatric, 4; neurologic, 2; various causes, 6; other cause, 2	72 patients included n patients not followed up NR Samples from 72 patients analysed 106 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Soki *et al*. (2010)[81]	Adults/children/neonates NR, age NR n male NR Patients with haematology malignancies with fever (126/162), no further details provided Limited details provided regarding patients receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 159 patients analysed 162 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Tsalik *et al.* (2010)[82]	Adults, 18 to 97, age 54.1 (range 18 to 97) 168/306 (54.9%) male n immunocompromised NR There were 69 subjects (22.5% of 306) who received at least one dose of antibiotic before blood was collected for culture or PCR Previous antibiotic exposure NR	Non-infected SIRS positive, 43 (14.1%); Sepsis, 184 (60.1%); Severe sepsis, 42 (13.7%); Septic shock, 37 (12.1%)	Sepsis was defined as SIRS with evidence of infection but no evidence of end-organ damage. Severe sepsis occurred in the presence of end-organ damage, which included metabolic damage, hematologic damage, pulmonary damage, or renal damage. Sepsis in the presence of hypotension, despite fluid challenge, or a blood lactate concentration of 4 mmol/litre was defined as septic shock.	Lung, 55 (18.0%); Urinary tract, 46 (15.0%); Skin, 41 (13.4%); Intra-abdominal, 25 (8.2%); Intravascular catheter, 16 (5.2%); Other, 32 (10.5%); Unknown, 91 (29.7%) Other symptoms NR	306 patients included n patients not followed up NR Samples from 306 patients analysed 306 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Wallet *et al.* (2010)[83]	Adults, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR - Antimicrobial prescription was prospectively recorded on the day of blood culture sampling Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR - patient status was defined according to SIRS criteria	The most frequent suspected site of infection was the respiratory tract: 66% (59/90) of samples with negative blood culture results, and 70% (7/10) of samples with positive blood culture results Other symptoms NR	72 patients included n patients not followed up NR Samples from 72 patients analysed 102 paired samples
Yanagihara *et al.* (2010)[84]	Adults/children/neonates NR, age NR 137/212 (65%) male Varied including immune deficiency [33/407 samples], tumour (51/407 samples) Of the pathogens detected by SeptiFast or blood culture, 40 were from patients who had been administered antibiotics and 32 of these 40 samples were from patients that had been administered antibiotics that matched the spectra of the antibiotics Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Sepsis was classified according to the ACCP/SCCM consensus conference criteria[2]	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 212 patients analysed 400 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Bravo *et al.* (2011)[85]	Adult, age 65.5 median (range 23-86) 33/53 (62%) male n immunocompromised NR None receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	severe sepsis or septic shock n=15	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 53 patients analysed 53 paired samples
Hettwer *et al.* (2011)[86]	Adults, age 62.3 (±18.1) 94 (61%) male n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	53.7% had severe sepsis or shock	At least 2/4 SIRS criteria. Patients also stratified according to procalcitonin and assessed against APACHE II and SOFA	Medical, non-pneumonogen n=55/153 (37%) Medical pneumonogen n= 63 (42%) Urogenital n= 17 (11%) Other n= 14 (9%) Procalcitonin, ng·ml: 14.4±42.3	211 patients recruited (153 with sepsis) PCR and blood culture available for 113 patients 113 paired samples
Josefson *et al.* (2011)[87]	Adults and children (range 14-98 years), age 67 (median) 607/1093 (56%) male n immunocompromised NR 36/200 pathogen detections in presence of antibiotics Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	1540 patients included 447 patients did not have results from at least one blood culture/PCR set not followed up Samples from 1093 patients analysed 1141 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Lucignano *et al.* (2011)[88]	Neonates and children, age NR n male NR n immunocompromised NR - 272/803 (33.9%) from Oncology, haematology, neonatology Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	The condition of sepsis was defined when a SIRS was in the presence of or a result of suspected or proven infection	Site of infection NR Clinical wards: heart surgery, n=323; haematology, n=272; cardiology, n=208. Others, Other symptoms NR	811patients recruited 8 not followed up Samples from 803 patients analysed 1553 paired samples
Obara *et al.* (2011)[89]	Adults (30 to 86), age 61.6 (range 30 to 86) 35/54 (64.8%) male n immunocompromised NR - 21/54 (38.9%) haematology Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	At least two criteria of the SIRS	Site of infection NR Other symptoms NR	54 patients included n patients not followed up NR Samples from 54 patients analysed 78 paired samples
Vrioni *et al.* (2011)[90]	Adults/children/neonates NR, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 33 patients analysed 33 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Alvarez *et al.* (2012)[91]	Adults, mean age 64.9 55 (54.5%) male n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	All had severe sepsis or septic shock	Dellinger *et al.* 2008[156] and by Levy *et al.* 2003[157]	Emergency abdominal surgery: BC=9, SF=11; elective abdo surgery, BC=2, SF=2; pneumonia, BC=0, SF=4; pancreatitis, BC=7, SF=1; CNS lesion, BC=5, SF=9; polytrauma/head, BC=20, SF=4; heart surgery, BC=2, SF=20; vascular surgery, BC=1, SF=1; pneumonectomy, BC=1, SF=0 Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 102 patients analysed n paired blood samples NR
Grif *et al.* (2012)[92]	Adults/children/neonates NR, age 55.6 42/61 (69%) male Adults/children NR n immunocompromised NR 56/61 (91.8%) receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	At least 2 SIRS criteria - sepsis not defined	Site of infection NR Other symptoms NR	61 patients included n patients not followed up NR Samples from 61 patients analysed 71 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Guido *et al.* (2012)[93]	Adults, age 66.1 median (range 23-82) 103/166 (62%) male None immunocompromised None receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Febrile neutropenia - sepsis not defined	Site of infection NR Other symptoms NR	166 patients included n patients not followed up NR Samples from 166 patients analysed 166 paired samples
Lodes *et al.* (2012)[94]	Adults 20 to 88 years, age 63.1 (14.1) 74/104 (71.1%) male n immunocompromised NR - malignant neoplasm 38 (36.5%) 79.7% of all blood samples were taken under antibiotic therapy and 41.9% were taken under antifungal therapy. Previous antibiotic exposure NR	Severity Suspected, severe, shock NR Severity of disease NR.	Sepsis was classified according to the ACCP/SCCM consensus conference criteria[2]	All abdominal Main diagnosis by surgery: Malignant neoplasm, 38 (36.5%); Peritonitis, 23 (22.1%); Hepatorenal syndrome, liver failure, liver cirrhosis, 14 (13.5%); Haemorrhage, 7 (6.7%); Ischaemia, 6 (5.8%); Pancreatitis, 5 (4.8%); Urosepsis, 2 (1.9%); Gall bladder perforation, choledocholithiasis, 2 (1.9%); Hernia incarceration, 2 (1.9%); Others, 5 (4.8%)	104 patients included n patients not followed up NR Samples from 104 patients analysed 148 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Mauro *et al.* (2012)[95]	Adult and children, age 5 to 68, age Range 5 to 68 41/79 (51.9%) male All immunocompromised All but 4 patients had blood culture drawn before starting antimicrobial therapy Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Suspected bloodstream infections and at least 2 criteria for SIRS	Site of infection NR 21 acute lymphoblastic leukaemia, 2 Wilms' tumour, 3 hepatocellular carcinoma, 2 allogeneic stem cell transplantations, 18 non-Hodgkin's lymphoma, 4 colon cancer under chemotherapy, 2 ovarian cancer, 1 Leishmania visceral, and 32 exposure to glucocorticoids for autoimmune disease	79 patients included n patients not followed up NR Samples from 79 patients analysed 79 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Pasquilani *et al.* (2012)[96]	Adults (20 to 99), age Median 73 (range 20 to 99) 215/391 (55%) male 17 (4%) immune deficiency 191/391 (48.8%) had been receiving antibiotic treatment for ≥24 h Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Suspected sepsis, suspected of having SIRS caused by bacterial or fungal infection	Site of infection NR History of CV disease, 88 (22%); Malignancy, 70 (18%); Dementia, 46 (12%); Chronic lung disease, 44 (11%); Diabetes, 41 (10%); Chronic renal failure, 28 (7%); Immune deficiency, 17 (4%); Chronic liver disease, 16 (5%); Gangrene, 3 (1%)	391 patients included n patients not followed up NR Samples from 391 patients analysed 391 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Rath *et al.* (2012)[97]	Adults, age 27 to 70, age 52.6 (10.9); range 27 to 70 72 (64.6%) male n immunocompromised NR – all liver transplant patients Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Patients with suspected sepsis according to the criteria of the ACCP/SCCM[17]	Site of infection NR Cirrhosis (Alcoholic, 21 (26.5); Infectious [hepatitis B/C], 17 (21.5); NASH, 4 (5.0); Other [autoimmune, unknown], 11 (13.9)); Hepatocellular carcinoma, 12 (15.1); Primary sclerosing cholangitis, 7 (8.8); Acute liver failure, 4 (5.0); Liver cysts, 3 (3.7); Malignancy, 48 (60.7); Abdominal infection, 27 (29.6); Abdominal organ perforation, 12 (13.1); Colon ischemia, 4 (4.3)	170 patients included n patients not followed up NR Samples from 170 patients analysed 225 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Tschiedel *et al.* (2012)[98]	Adults and children, median age 6 (range 0 to 24), age Median 6 (range 0 to 24) 37/75 (49%) male 64 samples were drawn from immunosuppressed patients (58%) 97 samples were drawn from patients (88%) under antibiotic treatment at time of sample taking Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR 87% of patients suffered from severe underlying disease such as: organ transplantation, malignant illnesses, cystic fibrosis, pulmonary hypertension, cardiac vitium, spinal muscular atrophy, renal insufficiency with dialysis 14 samples (13%) were taken from previously healthy patients	75 patients included n patients not followed up NR Samples from 75 patients analysed 110 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Herne *et al.* (2013)[99]	Adults, age 58 (range 20-81) 61/144 (42%) male n immunocompromised NR - haemoto -oncology study cohorts 143/144 (99%) receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	100% severe sepsis	Clinically suspected sepsis or septic shock or severe infection without known etiologic agent - sepsis not defined	Acute pneumonia (43%), central venous catheter associated bloodstream infection (11%), acute peritonitis (12%), septic endocarditis (10%), acute pancreatitis (10%), and acute urinary tract infection (4%). A total of 108 (75%) patients had multifocal infection with concurrent diagnoses and polymicrobial aetiology.	n patients recruited NR n patients not followed up NR Samples from 144 patients analysed 160 paired samples
Kasper *et al.* (2013)[100]	Neonates of very low birth weight, age NR (However, for blood culture+ sepsis and blood culture- clinical sepsis group age range from 23.3 to 30.1 weeks) n male NR n immunocompromised NR - neonates (mean [assumed] birth weight 818 ±242g) None receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	46 patients included n patients not followed up NR Samples from 46 patients analysed n paired blood samples NR

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Paolucci *et al.* (2013)[101]	Adults and children Age NR - 23 children, 178 adults n Male NR n immunocompromised NR- included 2 cases of autoimmune thrombocytopaenia Antibiotics prior to blood sample None Previous antibiotic exposure NR	NR	NR	Haematological malignancies (105 acute myeloid leukaemia, 23 acute lymphoblastic leukaemia, 34 lymphoma, 15 multiple myeloma, and 8 chronic myeloproliferative disorders), severe aplastic anaemia (4 patients), solid tumours (9 patients), or other disorders (2 cases of autoimmune thrombocytopaenia, 1 case of haemophagocytic lymphohistiocytosis).	n patients recruited NR n patients not followed up NR n patients analysed 201 (339 episodes) n paired blood samples 437
Rodrigues *et al.* (2013)[102]	Adults, age SF: 63 (46 to 75) BC: 66 (39 to 85) 31/46 (67%) Male n immunocompromised NR Antibiotics prior to blood sample none Previous antibiotic exposure none	Septic Shock SF:9/17 (53%); BC:16/29 (55%)	NR	NR	n patients recruited NR n patients analysed 46 n paired blood samples NR

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Avolio *et al.* (2014)[103]	Adults, age NR n male NR None immunocompromised Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Suspected BSIs and at least two criteria of the systemic inflammatory response syndrome	Site of infection NR Other symptoms NR	830 patients included 305 cases did not have a blood culture assay (not requested by clinician if patients under antibiotic treatment at time of blood sampling, or previous blood culture negative such as long term critically ill patients Samples from 525 patients analysed 525 paired samples
Burdino *et al.* (2014)[104]	Adults, age NR n male NR n immunocompromised NR - 10.5% HIV infection 89% receiving empirical antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Sepsis as defined by a systemic inflammatory response syndrome (SIRS) with suspected bacterial or fungal infections	Site of infection NR Other symptoms NR	1024 patients included n patients not followed up NR Samples from 1024 patients analysed 1186 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Mancini *et al.* (2014)[105]	Adults, mean age 48.6 152/228 (67%) Male n immunocompromised NR - 100% haemotological (55.7% with acute myeloid leukemia) Antibiotics prior to blood sample NR Previous antibiotic exposure NR	NR	Sepsis was defined according to Dellinger *et al.* 2013[16]	Site of infection NR Other symptoms NR	n patients recruited NR Six episodes in the prospective cohort and four in the retrospective were excluded for incomplete compilation of the study records. Retrospective cohort,134 episodes in 115 patients; prospective cohort, 131 episodes in 113 patients analysed n paired blood samples NR
Markota *et al.* (2014)[106]	Adults, age 59.5 (14.8) 38/57 (66.7%) male n immunocompromised NR 39/57 (61.9%) receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	All had severe sepsis or septic shock Severity of disease Entire cohort: mean admission APACHE score 25 (±7.6)	Sepsis was defined according to Dellinger *et al.* 2008[156]	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 57 patients analysed 63 paired samples
Ozkaya-Parlakay *et al.* (2014)[107]	Children age 1 month to 17 years, age 2.71 (4.11) years (73.4% under 2 years of age) 43/69 (62.3%) male None (no immune deficiency) Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR - patients with two signs of SIRS included	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 69 patients analysed 79 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Schaub *et al.* (2014)[108]	Adults ≥18 years, age Median 64 56/110 (60%) male 15/110 (14%) Immunosuppression (drug-induced or HIV) Antibiotic therapy prior to presentation at ED had been started in 16 (15%) patients Previous antibiotic exposure NR	Sepsis without organ dysfunction in 61 patients (77%), severe sepsis in 13 (17%) and septic shock in 5 (6%).	Sepsis and its severity were defined according to the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference.[157] Patients were only considered to have sepsis if they had both SIRS and infection	Pulmonary 38 (35%); Urogenital 19 (17%); Abdominal 8 (7%); Musculoskeletal 3 (3%); Skin 7 (6%); Ear-nose-throat 5 (5%); Other 3 (3%); Systemic 5 (5%); No focus found 3 (3%) Diabetes, 26 (24%); renal impairment, 22 (20%); immunosuppression, 15 (14%)	n patients recruited NR n patients not followed up NR Samples from 110 patients analysed 205 paired samples
Sitnik *et al.* (2014)[109]	Adults, age 49.7 (24.8) 74/114 (64.9%) male Oncology patients (38/114), no further details provided Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Sepsis was classified according to the ACCP/SCCM consensus conference criteria (Bone *et al.*[4])	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 114 patients analysed 114 paired samples
Barbanti *et al.* (2015)[122]	Adults/children, NR Age NR n Male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	NR	NR	NR	n patients recruited NR n patients not followed up NR n patients analysed 491 n paired blood samples NR

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Calitri *et al.* (2015)[111]	Children and neonates (8 preterm newborns) Median age 6.8 (IQR: 2.7 to 13.1) 183/289 (63.3%) male n immunocompromised NR Antibiotics prior to blood sample NR (however, high rate of patients received antibiotic and or antifungal treatment at time of sampling Previous antibiotic exposure NR	NR	International Pediatric Sepis Consensus[158]	NR	n patients recruited NR n patients not followed up NR n patients analysed 289 (545 episodes) n paired blood samples NR
Idelevich et al. (2015)[112]	Adults Mean age 52.4 (SF, 50.4 (14.4); BC, 54.4 (15.2)) 89/150 (59.3%) male (SF, 45/74 (60.8%); BC, 44/76 (57.9%)) n immunocompromised NR Antibiotics prior to blood sample 150/150 (100%) Previous antibiotic exposure NR	NR	Sepsis was defined according to the SCCM/ACCP consensus conference guidelines of 1992[4]	Acute myeloid leukemia SF=33 (44.6), BC=42 (55.3); Acute lymphoblastic leukemia SF=8 (10.8), BC=12 (15.8); Multiple myeloma SF=12 (16.2), BC= 11 (14.5); Non-Hodgkin lymphoma SF=18 (24.3), BC= 6 (7.9); Chronic myeloid leukemia SF=1 (1.4), BC=3 (3.9); Myelodysplastic syndrome SF=1 (1.4), BC= 1 (1.3); Others SF=1 (1.4), BC= 1 (1.3)	n patients recruited NR n patients not followed up NR n patients analysed 150 n paired blood samples 253

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Tafelski *et al.* (2015)[114]	Adults (range 47-74), age blood culture+SeptiFast: 67 (median);blood culture: 59 (median) blood culture+SeptiFast: 26 (63%) male; blood culture: 24 (65%) male blood culture+SeptiFast: 6/41 (15%); blood culture: 6/37 (16%) immunocompromised Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Septic shock blood culture+SeptiFast: 20/41 (49%) blood culture: 25/37 (68%) Median SAPS II on admission blood culture+SeptiFast: 40 (IQR 32-50) blood culture: 47 (IQR 34-65)	Sepsis defined as suspected or proven infection causing systemic inflammation with at least two of the following: (i) leucocyte count <4 or >12 /nl; (ii) body temperature <36 C or fever >38 C; (iii) tachypnoea >20/min or hyperventilation (paCO2 <32 mmHg); (iv) tachycardia >90 bpm.	Abdominal blood culture+SeptiFast: 15/41(37%) blood culture: 8/37 (22%) Pulmonary blood culture+SeptiFast: 26/41(63%) blood culture: 29/37 (78%) Other symptoms NR	100 patients included 22 (unable to provide informed consent) not followed up Samples from 78 -blood culture+SeptiFastI: 41; blood culture: 37 patients analysed 78 paired samples
Warhurst *et al.* (2015)[113]	Adults and children age of ≥16 years, age Median 58 (44 to 68) 553/795 (60%) male n immunocompromised NR 788/795 (85%) receiving antibiotic treatment at time of blood collection Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Suspicion of bloodstream infection was based on the development of two or more SIRS criteria as defined by Levy *et al.* 2003[157]	Site of infection NR Other symptoms NR	795 patients included n patients not followed up NR Samples from 795 (922 episodes) patients analysed n paired blood samples NR
SINGLE INDEX TEST STUDIES - SEPSITEST					

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Wellinghausen *et al*. (2009)[48]	173 adults and 14 children younger than 18 years, age NR n male NR n immunocompromised none Antibiotics prior to blood sample Eight of the thirteen patients received broad-spectrum antimicrobials before sampling Previous antibiotic exposure NR	148 patients (79.1%) were ICU patients fulfilling the criteria for SIRS or sepsis and 39 patients (20.9%) were haematological patients with neutropenic fever	NR	NR	n patients recruited NR n patients not followed up NR Samples from 187 patients analysed 342 paired samples
Loonen *et al*. (2014)[116]	Adults, Pos blood culture, , age 68.9 (17.3); neg blood culture, , age 60.4 (18.0) Pos blood culture, 17/125 (13.6%) male; neg blood culture, 57/125 (45.6%) male n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	140 patients included 15 - alternative diagnosis without infection not followed up Samples from 125 patients analysed n paired blood samples NR

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Nieman et al. (unpublished)[115]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
SINGLE INDEX TEST STUDIES - IRIDICA					
Bacconi *et al*. (2014)[49]	Adults ≥18 years, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	Patients with suspected sepsis enrolled	Site of infection NR Other symptoms NR	331 patients included n patients not followed up NR Samples from 331 patients analysed 331 paired samples
Delco-Volante *et al*. 2015[120]	Neonates (<28 days old), age NR n male NR n immunocompromised NR - neonates None - before the initiation of antibiotics Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR n patients analysed NR 81 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Vincent *et al*. (in press)[121]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Metzgar (unpublished)[119]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TWO INDEX TEST STUDIES – SEPTIFAST AND SEPSITEST					
Leitner *et al*. (2013)[117]	Adults/children/neonates NR, age NR n male NR n immunocompromised NR Antibiotics prior to blood sample NR Previous antibiotic exposure NR	Suspected, severe, shock NR Severity of disease NR	NR	Site of infection NR Other symptoms NR	n patients recruited NR n patients not followed up NR Samples from 57 patients analysed 75 paired samples

Author (year)	Adults/children/neonates, mean (SD) age, n/N (%) males, n immunocompromised, n exposed to antibiotics prior to blood sample collection, previous antibiotic exposure (history)	Sepsis – n (%) Suspected, Severe, Shock; severity of disease	Definition of sepsis	Sites of infection, Other symptoms	N patients recruited/included, not followed up, analysed; n paired blood samples
Schreiber *et al*. (2013)[118]	Adults, age Median 64 (IQR 51 to 70) 10/50 (20%) male n immunocompromised NR - 2/50 (1%) had bone marrow transplant Antibiotics prior to blood sample NR 36/50 (72%) had received antibiotic treatment at recruitment	Sepsis, 10 (20%); severe sepsis, 13 (26%); septic shock, 27 (54%) Severity of disease Entire cohort: SAPS II, median 41 (IQR 33 to 49)	Diagnosis of sepsis according to the sepsis criteria of the German Sepsis Competence Network[159]	Site of infection NR Reasons for admission: Surgical 20 (40%) (Abdominal, 7; Chest, 6; Trauma, 7); Medical, 24 (48%) (Pneumonia, 15; Pancreatitis/Cholangitis, 2; Bone marrow transplant, 2; Unknown focus, 5)	n patients recruited NR n patients not followed up NR Samples from 50 patients analysed n paired blood samples NR

Table 58: Characteristics of the index and reference tests

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contamin ants included?
SINGLE INDEX TEST STUDIES - SEPTIFAST								
Raglio *et al.* (2006)[60]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: NR When samples drawn NR	Unclear	NR	blood culture in conjunction with clinical adjudication Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Sample	Included
Bingold *et al.* (2007)[61]	LightCycler SeptiFast Test - MGRADE not reported 3 ml K-EDTA venous or arterial blood When samples drawn NR	Unclear	NR	blood culture with swabs from suspicious sites for microbiological diagnostics Sampling method NR Culture method NR	NR	NR	Samples	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Klemm *et al.* (2007)[62]	LightCycler SeptiFast Test - MGRADE not reported 2 x 3.5 ml EDTA-blood Reference standard and index tests performed on blood samples drawn at the same time	Unclear	Same day	blood culture and procalcitonin as a clinical marker of sepsis 2 x 10ml BACTEC BD	NR	NR	Patients	NR (4 samples contaminated with dermal Streptococci deliberately not reported by SeptiFast interpretive software)
Lodes *et al.* (2008)[63]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: NR When samples drawn NR	Unclear	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Sample	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Louie *et al.* (2008)[46]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: 3 mL EDTA for PCR testing When samples drawn NR	Unclear	NR	blood culture plus clinical chart review Sample: Whole blood Site: NR Volume: NR - the PCR sample was collected after a sample was drawn for blood culture NR	NR	NR	Patients	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Mancini *et al.* (2008)[45]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: at least 1.5ml K-EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: at least 20ml (average of 25ml inoculated into aerobic and anaerobic bottles) BacT/ALERT 3D automated blood culture system, with monitoring of carbon dioxide production within each bottle every 10 min 24 h per day. All bottles signalled as positive were removed from the instrument, and an aliquot was taken for Gram stain and culture on solid media. Sensitivity to antibiotics were performed with the VITEK 2 system	NR	Seven days /week (from 8am to 7pm)	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Vince *et al.* (2008)[64]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: NR When samples drawn NR	Unclear	NR	blood culture in conjunction with clinical adjudication Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Samples	NR
Dark *et al.* (2009)[65]	LightCycler SeptiFast Test - MGRADE not reported NR When samples drawn NR	Unclear	NR	blood culture with input from results of other cultures Sampling method NR Culture method NR	NR	NR	Pathogens	NR (3 detected in blood culture but not in SeptiFast assigned as true negatives)

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Dierkes *et al.* (2009)[66]	LightCycler SeptiFast Test - MGRADE not reported Whole blood NR Reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	blood culture concordance. Clinical data were extracted by chart review and by the data provided for the test application Whole blood NR 2 x 10 ml bottles BACTEC 9240. Both aerobic and anaerobic blood culture bottles were inoculated directly with 10 ml blood each and delivered to the microbiology department together with the aliquot for analysis with the SeptiFast. Blood cultures were incubated for 7 days.	NR	Seven days/week (Monday to Friday from 8AM to 7PM; Saturday, Sunday and holidays from 9AM to 4PM)	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Gimeno *et al.* (2009)[67]	LightCycler SeptiFast Test. MGRADE not reported Sample: Whole blood Site: Venous or catheter Volume: 3ml Reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	Traditional blood cultures (aerobic and anaerobic)	NR	NR	Samples	NR
Lehmann *et al.* (2009)[68]	LightCycler SeptiFast Test - MGRADE not reported Sample: NR Site: NR Volume: NR Unclear if reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	The study was not designed for method comparison, therefore multiple BC tests per episode were allowed. blood culture was performed using BACTEC or BacT/ALERT	NR	NR	Incomplete diagnostic data reported	Incomplete diagnostic data reported
Lodes *et al.* (2009)[69]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: NR When samples drawn NR	Unclear	NR	blood culture in conjunction with clinical adjudication Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Palomares *et al.* (2009)[70]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: 3 ml in EDTA bottles Reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	blood culture in conjunction with clinical adjudication Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Sample	Included
Paolucci *et al.* (2009)[71]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: peripheral venous Volume: 1.5ml When samples drawn NR	Yes	NR	blood culture plus clinical. BSI was confirmed by the presence of clinical signs of infection or additional microbiological data. Sample: Whole blood Site: peripheral venous Volume: 1.0ml blood cultures were performed according to the Clinical and Laboratory Standards Institute (CLSI) protocol. No further detail reported.	NR	NR	Patients	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Varani *et al.* (2009)[72]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: adults, peripheral veins; children, central venous catheter Volume: K-EDTA 3ml was sampled and processed for patients who weighed 45 kg and 1.5 ml for those who were <45 kg Reference standard and index tests performed on blood samples drawn at the same time	Unclear (mixed based on weight - immunocompromised patients)	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC system and processed according to the Clinical and Laboratory Standards Institute	NR	NR	Febrile episodes	included
von Lilienfeld-Toal., *et al.* (2009)[73]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: Venous or catheter Volume: 1.5 ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	No. 1ml (adults)	NR	BC was performed using the BACTEC system	NR	NR	Pathogen	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Westh *et al.* (2009)[74]	SeptiFast lys kit, the SeptiFast prep kit, and the LightCycler SeptiFast kit, all MGRADE Sample: Whole blood Site: Venous Volume: Used 1.5 ml for assay (drawn 5 ml) Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture in conjunction with clinical adjudication. Identification of microorganisms from a suspected infectious focus within 48 h of the episode was used to resolve discrepancies in the results Sample: Whole blood Site: Venous Volume: 8-10 ml for each aerobic and anaerobic bottle for each system blood culture was performed using the BACTEC or BacT/ALERT system. Each blood culture was performed in a pair of aerobic/anaerobic bottles. Blood for one or two additional blood culture sets was collected from each patient within a 24-h period and included in episode evaluation.	NR	NR	Pathogen	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Berger *et al.* (2010)[75]	LightCycler SeptiFast Test - MGRADE not reported Modified DNA extraction for very low birth weight infants protocol to decrease blood volume requirements to 1.0 ml When samples drawn NR	No (0.1 ml; neonates)	NR	blood culture with clinical and laboratory signs of infection 0.1ml Sampling method NR	NR	NR	Patients	Included
Bloos *et al.* (2010)[76]	LightCycler SeptiFast Test - MGRADE not reported Whole blood (10ml) Venous 3ml from 10ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	No (3ml; adults)	Same day as severe sepsis suspected	blood culture and clinical/laboratory confirmation Whole blood (20ml) for conventional cultures Pair of blood cultures incubated at 37 degree Celsius and monitored for 8 days. Isolated microorganisms and their susceptibilities were determined by standard methods and criteria.	Clinical adjudicators reviewed the patient data and corresponding microbiological culture results of the presumed site of infection	NR	Samples	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Lamoth *et al.* (2010)[77]	LightCycler SeptiFast Test. MGRADE not reported Sample: Whole blood Site: venous Volume: 1ml Reference standard and index tests performed on blood samples drawn at the same time	No, 3ml (adults)	For SF assays, DNA was extracted from the EDTA whole-blood tubes within 48 to 72 h after sampling	BC was performed using the BACTEC system.	NR	NR	Episodes	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Lehmann *et al.* (2010)[78]	SeptiFast Prep Kit and the LightCycler - MGRADE not reported Sample: Whole blood Site: NR Volume: Used 1ml EDTA whole blood sample (9 ml was drawn for further PCR analysis) Reference standard and index tests performed on blood samples drawn at the same time	No (1 ml; adults)	NR	blood culture and clinical/laboratory confirmation A pair of aerobic/anaerobic blood culture bottles Also provides analysis for a constructed gold standard including blood culture and other microbiological tests (sens 0.83 and spec 0.93) Sample: Whole blood Site: NR Volume: 20 ul for pair of aerobic and anaerobic blood culture bottles blood culture was performed using the BACTEC system. All blood culture were processed using semi-automated blood culture systems according to the manufacturer's instructions. The blood culture system and the local laboratory software automatically registered time to positive blood culture.	A blood stream infection was defined as a positive blood culture result, obtained and analysed as set forth by the current DGHM procedures. Whether microorganisms identified by PCR represented true infection or contamination was evaluated retrospectively by taking into account the identity of the microorganism detected and by comparing PCR results with corresponding blood culture findings	NR (however labs were not 24/7)	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Maubon *et al.* (2010)[79]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: 1.5ml EDTA When samples drawn NR	Yes	NR	blood culture plus clinical - leukocyte count, C-reactive protein and procalcitonin measurement, two sets of bacterial and fungal blood cultures, urine culture, chest radiograph and, when appropriate, specific viral and fungal tests Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Patients	Unclear

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Reguerio *et al.* (2010)[80]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: venous or arterial draw Volume: 1.5ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: venous or arterial draw Volume: 10ml blood culture was performed using the BacT/ALERT system. Once flagged by the instrument for detectable growth, fluid was withdrawn for gram stain and appropriate agar-based culture plates. Isolated colonies were analysed either by an automated identification system Vitek II	NR	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Soki *et al.* (2010)[81]	SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: NR When samples drawn NR	Unclear	NR	blood culture - NR if with clinical or not Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC system. Blood culture sets (1 aerobic and 1 anaerobic bottle) were cultured	NR	NR	Sample	NR
Tsalik *et al.* (2010)[82]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: 1.5ml When samples drawn NR	Yes	NR	blood culture plus clinical Sample: Whole blood Site: NR Volume: the volume inoculated was not monitored blood culture was performed using the BacT/ALERT system plus BACTEC	NR	NR	Patients	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Wallet *et al.* (2010)[83]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: venepuncture, site NR Volume: EDTA 5ml, volume for DNA prep 1.5ml Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: venepuncture, site NR Volume: 10ml blood culture was performed using the BacT/ALERT system plus BACTEC	NR	NR	Pathogens	included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Yanagihara *et al.* (2010)[84]	SeptiFast-Lys and Prep MGRADE kits Sample: Whole blood Site: NR Volume: Used 1.5 ml for assay (drawn 10 ml) Reference standard and index tests performed on blood samples drawn at the same time	Yes	Blood for DNA Detection Kit was stored at -20°C for up to 72 hours before testing	blood culture in conjunction with clinical adjudication. When the result of blood culture analysis was positive, the sample was identified using each site's identification system Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC and BacT/ALERT system. blood culture bottles whose results were positive were sent them to one commercial laboratory to confirm the validation of the identification microorganisms	NR	NR	Sample	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Bravo *et al.* (2011)[85]	LightCycler SeptiFast Test MGRADE Whole blood Venous 1.5ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Whole blood Venous Paired 10 ml bottles BACTEC 9420 blood culture. A pair of bottles for aerobic and anaerobic bacteria and an additional bottle for fungal recovery. The blood cultures were incubated for a maximum of 7 days. Each bottle was inoculated with 10 ml of blood. The different sets of blood cultures were obtained at intervals of 30 min. Direct smear examination (Gram staining) was performed from positive blood cultures as soon as detected, Obtained at 30 min intervals	The significance of either the isolation of a potentially contaminating microorganism in a single set of blood cultures or the detection of CoNS DNA in blood by the SeptiFast assay was judged on the basis of clinical grounds	NR	Episodes	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Hettwer *et al.* (2011)[86]	LightCycler SeptiFast Test - MGRADE not reported Whole blood Venous 10 ml EDTA During the same venous puncture as blood culture	Unclear	Whilst blood culture/ SeptiFast samples collected at same time, SeptiFast measurements obtained 5 months after all blood culture results were available	blood culture with microbiological data and clinical outcome Whole blood 2 x 10ml bottles BacT/ALERT analysed at Institute of Medical Microbiology. When an aerobic and/or anaerobic bottle returned a positive result, Gram stain procedure and assay culture were performed according to standardized procedures.	NR	NR	Patients	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Josefson *et al.* (2011)[87]	SeptiFast Lys Kit MGRADE and the MagNA Lyser Sample: Whole blood Site: Venous (for all samples) Volume: 1.5ml EDTA whole blood sample Reference standard and index tests performed on blood samples drawn at the same time	Yes	Whole blood was stored for a maximum of 4 h at room temperature, or up to 3 days at +4°C, or 3 months at −70°C prior to DNA preparation	blood culture and clinical/laboratory confirmation One BACTEC Plus Aerobic/F bottle and one BACTEC Plus Anaerobic/F bottle. Sample: Whole blood Site: Venous (for all samples) Volume: 8-10 ml for each aerobic and anaerobic bottle After transport at room temperature, the bottles were placed in a BACTEC 9240 incubator, with monitoring for pH changes every 10 min for 6 days. All signalling bottles were opened and an aliquot was taken for microscopy after Gram staining, culture on solid media, and further analyses for species designation	A positive PCR result was considered to be fully supported when an identical microorganism was isolated in the blood culture of the same blood culture/PCR set.	NR	Patients	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Lucignano *et al.* (2011)[88]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: peripheral or central venous line Volume: ≥1.5ml paired blood samples for SeptiFast Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture plus clinical (vital signs) and lab variables (leukocyte count, C-reactive protein, microbiological evidence of infection focus) Sample: Whole blood Site: peripheral or central venous line Volume: 0.5 to 10ml depending on whether aerobic or anaerobic bottle blood culture was performed using the BACTEC system. The bottles were then incubated at 35°C in BACTEC 9240/9120 blood culture system (BD Diagnostics) cabinets for 8 days. In case of positivity, Gram staining and culture on solid medium were performed; definitive organism identification and antibiotic susceptibility were determined with accredited routine laboratory methods (Vitek 2 system [bioMe´rieux, Durham, NC] or Phoenix [BD Diagnostics] system).	The condition of sepsis was defined when a SIRS was in the presence of or a result of suspected or proven infection (8), ascertained by the microbiology routine team, which addressed the final interpretation of the results (contaminants versus pathogens) on the basis of type of microbe, time to positivity (TTP), number of positive blood cultures for the same microbe, and patient data provided by clinicians.	NR	Samples	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Obara *et al.* (2011)[89]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: 1.5ml K-EDTA for the molecular method Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture - NR if with clinical or not Sample: Whole blood Site: NR Volume: 5ml blood culture was performed using the BACTEC system. After cultivation, the strain was identified by WalkAway 96 SI system and auto scan-4	NR	NR	Samples	Included
Vrioni *et al.* (2011)[90]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: NR When samples drawn NR	Unclear	NR	blood culture in conjunction with clinical adjudication Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Patients	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Alvarez *et al.* (2012)[91]	LightCycler SeptiFast Test - MGRADE not reported Sample: NR Site: NR Volume: NR Unclear if reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	Blood culture, tracheal aspirate, urine, surgical wounds, intravaneous catheters, and other sources. No further detail reported	NR	NR	No diagnostic data reported	No diagnostic data reported
Grif *et al.* (2012)[92]	LightCycler SeptiFast Test MGRADE Whole blood Venous 1.5ml processed from 5ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	Samples collected every day except weekends and testing done immediately	blood culture alone and blood culture with other microbiological cultures Whole blood Venous 20 ml BacT/ALERT 3D 2 bottles incubated for max 5 days at 37 degrees. From all bottles signalled as positive, microorganisms were isolated and identified according to standard laboratory methods	NR	NR (but samples collected every day except on weekends and testing done immediately with both assays)	Samples	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Guido *et al.* (2012)[93]	LightCycler SeptiFast Test MGRADE Whole blood Venous 1.5 ml K-EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Whole blood Venous 10 ml BacT/ALERT 3D 2 bottles. All bottles signalled as positive were removed from the instrument, and an aliquot was taken for Gram stain and culture on solid media for subsequent analysis. Identification and determination of sensitivity to antibiotics were performed with the VITEK 2 system		NR (but positive blood culture bottles from automated blood culture system removed between 8am and 7pm for further analysis)	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Lodes *et al.* (2012)[94]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: 1 mL EDTA for assay (a pair of aerobic/anaerobic blood culture bottles - 9ml whole blood collected by EDTA for further PCR analysis) Reference standard and index tests performed on blood samples drawn at the same time	No (1 ml; adults)	NR	blood culture alone plus clinical (text suggests clinical diagnosis) Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC system	NR	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Mauro *et al.* (2012)[95]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: 1.5ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: NR BACTEC 9240 - 1 set of blood cultures (aerobic/anaerobic and fungal). When the blood culture gave a positive signal, Gram staining was carried out. An aliquot of positive blood culture was plated onto solid media and incubated for 24/48 h, and identification was carried out with a Vitek 2 system	NR	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Pasquilani *et al.* (2012)[96]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: 1.5ml K-EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC system. All bottles flagged positive were removed from the instrument, and an aliquot was taken for Gram stain and culture on solid media for subsequent analysis. Identification and determination of sensitivity to antibiotics were performed with conventional methods and with the Phoenix automatic system	Microorganisms detected by SeptiFast were considered to be pathogens if the results of the DNA kit coincided with the results of the blood culture analysis.	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Rath *et al.* (2012)[97]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: venepuncture or central venous catheter Volume: 3ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: venepuncture or central venous catheter Volume: 8 to 10ml per bottle blood culture was performed using the BACTEC system	NR	NR	Samples	Included
Tschiedel *et al.* (2012)[98]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: 1.5ml Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC system	NR	NR (however labs open until 11pm Monday through Friday)	Samples	included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Herne *et al.* (2013)[99]	LightCycler SeptiFast Test MGRADE Whole blood Venous 1.5ml from 3 ml K2EDTA All blood culture/SeptiFast samples collected within 12 hours apart	Yes	Perform SeptiFast from 8 am to 4 pm only, 7 days of the week	blood culture alone and blood culture with clinical and other microbiological cultures Whole blood Venous >2 X 10ml bottles BacT/ALERT 3D At least 2 sets of 10ml paired bottles 0.5- 1hr apart during the acute febrile episode. Blood cultures for aerobes and anaerobes were incubated up to 7 days, those for fungi for up to 11 days. In case of positive blood cultures, microorganisms were identified according to standard laboratory procedures: (1) Gram staining, (2) subculture on non-selective and selective agar media according to the results of gram staining, (3) identification of pathogen by immunological, biochemical and enzymatic tests, and (4) susceptibility testing by disk diffusion test and/or gradient method for MIC detection accordingly to the identified pathogen and laboratory protocol.	Defined all cases in which both method gave positive results as well as all cases in which a positive result in either SeptiFast or blood culture was considered clinically relevant as true positive results	Seven days/week (SeptiFast assay performed from 8am to 4pm only)	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Kasper *et al.* (2013)[100]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: 0.1 to 0.7 ml (max) blood sample into 0.8 ml k3EDTA vacutainers Reference standard and index tests performed on blood samples drawn at the same time	No (0.1 to 0.7 ml; neonates)	Samples analysed immediately or stored at -20C and processed next day	blood culture plus clinical Sample: Whole blood Site: NR Volume: 0.5 to 1.0ml blood culture was performed using the BacT/ALERT system. Only aerobic paediatric bottle was inoculated and incubated for 7 day (due to limited blood volumes) by standard microbiological procedures	NR	NR	Patients	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Paolucci *et al.* (2013)[101]	LightCycler SeptiFast Test. MGRADE not reported Sample: NR Site: NR Volume: NR Reference standard and index tests performed on blood samples drawn at the same time	No, 3ml (adults)	Blood samples intended for PCR analyses arriving to the laboratory after 12:00 p.m. were stored at 4 °C until the next PCR session was programmed	BC was performed using the BacT/ALERT system.	NR	Blood cultures were accepted for automated incubation from 8:00 a.m. to 7:00 p.m. (Monday to Saturday). PCR was performed once per day from Monday to Friday (samples were received by 12:00 p.m.). Blood samples intended for PCR analyses arriving to the	Episodes	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Rodrigues *et al.* (2013)[102]	LightCycler SeptiFast Test - MGRADE not reported Sample: NR Site: NR Volume: NR Unclear if reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	BC was performed using the Bac/Talert system	NR	NR	Incomplete diagnostic data reported	Incomplete diagnostic data reported

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Avolio *et al.* (2014)[103]	LightCycler SeptiFast Test MGRADE 1.5 mL of whole blood was collected in sterile EDTA-KE. Reference standard and index tests performed on blood samples drawn at the same time	Yes	SeptiFast assays were performed once daily for samples collected in the previous 24 h	blood culture and clinical/laboratory confirmation A blood culture bottle of 20 mL of blood either by venepuncture or from an IV access device. BacT/ALERT 3D automated system(bioMe´rieux) All instances in which a maximum of 3 sets (6 bottles) of blood cultures for patient, obtained during a 24-h period and arrived simultaneously at laboratory was included When aerobic/anaerobic bottles gave a positive signal, Gram staining was carried out	Microorganisms detected by SeptiFast were considered to be pathogens if results coincided with those of blood culture and/or in accordance of the American College of Clinical Pharmacy/Society of Critical Care Medicine Conference Committee definition of infection.	Seven days/week (Monday to Friday from 7:30AM to 5PM; Saturday and Sunday from 7:30AM to 12:30AM)	Pathogen	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Burdino *et al.* (2014)[104]	LightCycler SeptiFast Test MGRADE Whole blood Venous 1.5 ml EDTA Reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	blood culture with clinical and laboratory signs of infection. Clinical evidences, laboratory findings, and microbiological data combined with the identification of the same bacteria from other body sites as defined by the Weinstein algorithm were used to confirm pathogens versus irrelevant and/or contaminants for both blood culture and SeptiFast Whole blood Venous 8-10 ml Biomerieux blood culture bottles incubated at least 24-72 hours (for positive blood cultures) to 5 days for negative results	NR	NR (but assays performed daily with one or more runs with dedicated personnel)	Samples	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Mancini *et al.* (2014)[105]	LightCycler SeptiFast Test - MGRADE not reported Sample: Uncultured blood Site: NR Volume: 1.5 ml Unclear if reference standard and index tests performed on blood samples drawn at the same time	Yes	NR	BacT/Alert 3D blood culture system	NR	The molecular assay in the prospective cohort was organized assuring two daily sessions from Monday to Friday	Incomplete diagnostic data reported	Incomplete diagnostic data reported

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Markota *et al.* (2014)[106]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: peripheral veins Volume: NR but EDTA 5ml drawn Reference standard and index tests performed on blood samples drawn at the same time	Unclear	On same day if sampled before 6pm, next day of sampled after 6pm	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: peripheral veins Volume: 20 ml (10 ml inoculated in each aerobic/anaerobic bottle blood culture was performed using the BacT/ALERT system. All blood culture bottles signalled as positive were processed according to standard microbiology laboratory procedures	NR	SeptiFast assays were performed from 8am to 4pm on weekdays and from 8am to 1pm of Saturdays	Sample	NR
Ozkaya-Parlakay *et al.* (2014)[107]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: venepunctures, site NR Volume: NR Reference standard and index tests performed on blood samples drawn at the same time	Unclear	If possible, PCR was evaluated immediately, if not, stored at -20C	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: venepunctures, site NR Volume: Sampling method NR Culture method NR	NR	NR	Sample	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Schaub *et al.* (2014)[108]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: venepunctures, site NR Volume: NR but collected in EDTA 2.7ml tubes Reference standard and index tests performed on blood samples drawn at the same time	Unclear	The turnaround time of MRT-PCR results was calculated on the assumption that the MRT-PCR could be performed 24/7 and using the reported turnaround time of the assay of approximately six hours	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: venepunctures, site NR Volume: 25ml inoculated into an aerobic and anaerobic bottle blood culture was performed using the BacT/ALERT system	Patients were categorised as "true positive" or "true negative" for bacterial sepsis on the basis of the expanded reference standard e.g. conventional microbiological methods such as e.g. culture of blood/urine/sputum, rapid antigen testing in throat swabs [streptococcal rapid antigen test] or urine [Streptococcus pneumoniae, Legionella pneumophila])	NR (however, Positive blood culture bottles removed from automated system from 8am to 5pm)	Patients	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Sitnik *et al*. (2014)[109]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: NR Volume: 5 ml collected from each patient in sterile EDTA Tube (mechanical lysis on 3mL) Reference standard and index tests performed on blood samples drawn at the same time	No (3 ml; adults)	Blood samples stored at -20°C and multiplex PCR testing done twice per week	blood culture in conjunction with clinical adjudication Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC system. When a positive signal was obtained, Gram staining of the blood culture medium in the bottles was performed. Samples were platted onto blood agar, chromogenic agar and anaerobic blood agar. Identification of bacterial or fungal species as well as antibiotic sensitivity tests were then carried out using the Vitek II system and API 32 C for yeast.	NR	NR	Sample	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Barbanti *et al.* (2015)[122]	LightCycler SeptiFast Test. MGRADE not reported Sample: NR Site: NR Volume: NR Unclear if reference standard and index tests performed on blood samples drawn at the same time	Unclear	NR	BC was performed using the BacT/Alert system.	NR	NR	Samples	Included
Calitri *et al.* (2015)[111]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: Venous or catheter Volume: 1.5 ml EDTA Unclear if reference standard and index tests performed on blood samples drawn at the same time	Unclear	Samples were processed within few hours of collection for the SeptiFast test; however, BC collected on same day or if unavailable the nearest BC performed was recorded (±48h max from SF collection)	BC was performed using the BacT/Alert system. Each BC consisted of one bottle aerobic or anaerobic cultures, each innoculated with minimum blood required according to paediatric age group	NR	NR	Episodes	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Idelevich et al. (2015)[112]	LightCycler SeptiFast Test MGRADE Sample: Whole blood Site: peripheral veins in adults CVC in children Volume: 3ml ≤45kg, 1.5ml >45kg Reference standard and index tests performed on blood samples drawn at the same time	Yes	Samples for PCR analyses that arrived at the laboratory after 12 noon were stored at 4 °C and processed on the next day. The median time to the arrival of BC samples at the microbiological laboratory was 13.3 h (12.9 h in the study group and 13.6 h in the control group, p = 0.14). It took 14.8 h for whole-blood samples of the study group patients to arrive at the PCR department. This includes the arrival time at the microbiological laboratory and forwarding samples to the PCR department.	BC was performed using the BACTEC system. The BC was incubated for at maximum seven days.	NR	BC Mon to Fri 7:30am to 6:00pm, Sat 7:30am to 1:30pm; PCR Mon to Fri 7:30am to 6:00pm	Pathogen	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Tafelski *et al.* (2015)[114]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: Venous or arterial site Volume: 1.5ml for SeptiFast assay Reference standard and index tests performed on blood samples drawn at the same time	Yes	Samples were processed immediately for the SeptiFast test; however, in the control group, samples were stored at -80C for later analysis	blood culture plus MALDI-TOF MS with clinical adjudication Sample: Whole blood Site: Peripheral venous blood Volume: 10ml each for blood culture (anaerobic or aerobic bottle) blood culture was performed using the BACTEC system. Positive bottles were streaked onto a set of agar plates and subjected to direct Gram staining. Pathogens identified using MALDI-TOF MS or biochemical identification test (Vitek 2). Commercial procedures used for antimicrobial susceptibility testing (Vitek 2 or Etest)	For analysis of the test performance of the LightCycler SeptiFast PCR test, blood culture results were used as the diagnostic gold standard for detecting bacteraemia	5 days a week (6am to 6pm)	Samples	NR

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Warhurst *et al.* (2015)[113]	SeptiFast Test - MGRADE not reported Sample: Whole blood Site: Two separate sites (including one peripheral site) Volume: used 1.5ml for assay (drawn 20 ml) Reference standard and index tests performed on blood samples drawn at the same time	Yes	Blood for DNA Detection Kit was stored at 4°C for up to 72 hours before PCR analysis	blood culture in conjunction with clinical adjudication. Blood cultures entered the standard clinical pathway, and the results were returned directly to the clinical service at each centre Sample: Whole blood Site: Two separate site (including one peripheral site) Volume: Two blood samples of 20 ml from two separate sites blood culture was performed using BacT/ALERT system. Blood cultures entered the standard clinical pathway	NR	NR	Pathogens	Excluded

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
SINGLE INDEX TEST STUDIES - SEPSITEST								
Wellinghausen *et al.* (2009)[48]	SepsiTest Sample: Whole blood Site: NR Volume: 1 ml in duplicate for adults and 1 ml in single for children. Reference standard and index tests performed on blood samples drawn at the same time	Yes	Samples were sent for PCR from the local laboratory to the central study laboratory in Bremen within 2 days. BC were incubated at the local laboratories in automated BACTEC 9240 systems for up to 7 days.	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: 20ml for adults, 3 to 5ml for children blood culture was performed using the BACTEC system in adults and BACTEC PED system in children	Probable to true bacteremia was assigned if (i) a bacterial species or genus that was detected by PCR was also cultured from a specimen other than blood within 5 days before or after obtaining the blood sample or (ii) the species detected was a typical causative pathogen of the clinical scenario and no other causative pathogen was detected	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Loonen *et al.* (2014)[116]	SepsiTest Remnant whole blood 1ml When samples drawn NR	Yes	NR	blood culture plus MALDI-TOF MS and clinical/laboratory confirmation Remnant whole blood 1ml blood culture was performed using the BacT/ALERT system. two pairs of aerobic and anaerobic bottles were obtained and incubated for at least five days with a maximum of seven days	NR		Samples	included
Nieman et al. (unpublished)[115]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contamin ants included?
SINGLE INDEX TEST STUDIES - IRIDICA								
Bacconi *et al.* (2014)[49]	IRIDICA-PLEX-ID Hybrid Sample: Whole blood Site: venepuncture, arm Volume: 5 to 15ml Reference standard and index tests performed on blood samples drawn within 30mins	Yes	Kept at 4C within 30min collection	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: venepuncture, arm Volume: NR blood culture was performed using the BACTEC system/ The bottles were incubated and monitored for 5 days before being called negative. Positive blood cultures were removed immediately from the instrument, and a Gram stain was performed.	When PCR/ESI-MS-positive but culture-negative specimens were confirmed by repeat PCR/ESI-MS testing of additional replicate specimens and the confirmed detections were considered true positives		Samples	NR
Delco-Volante *et al.* 2015[120]	IRIDICA Sample: Whole blood Site: venepuncture Volume: 0.5ml Reference standard and index tests performed on blood samples drawn at the same time	No (0.5 ml; neonates)	NR	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: Sampling method NR Culture method NR	NR	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Vincent *et al.* (in press)[121]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Metzgar *et al*. (unpublished) [119]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TWO INDEX TEST STUDIES – SEPTIFAST AND SEPSITEST								

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Leitner *et al.* (2013)[117]	SeptiFast Lys Kit and the SeptiFast Prep Kit. MGRADE not reported Sample: Whole blood Site: Venous or indwelling peripheral or central catheter Volume: 6 ml in EDTA tubes Reference standard and index tests performed on blood samples drawn at the same time SepsiTest Sample: Whole blood Site: Venous or indwelling peripheral or central catheter Volume: NR but collected in 6 ml in EDTA tubes Reference standard and index tests performed on blood samples drawn at the same time	Unclear	Samples were tested in parallel with blood culture	blood culture and clinical/laboratory confirmation blood culture sets (blood cultures) consisting of three pairs of aerobic/anaerobic blood culture bottles. If the instrument reported a blood culture bottle positive, conventional biochemical identification methods and susceptibility testing were done. Sample: Whole blood Site: Venous or indwelling peripheral or central catheter Volume: NR blood culture was performed using the BACTEC system. The blood culture was incubated for at maximum seven days.	Bacterial pathogens were considered true positive if growing in at least one blood culture bottle. Potential skin contaminants were considered true positive only if the identical organism was growing in two or more blood culture bottles	NR	Samples	Included

Author (year)	Name of test, Sampling method, interval between index test and reference standard	CE Approved blood volume	Lab test performed on same day as sampling method	Name of reference standard, Sampling method, Culture methods	Definition of true positive: Index test/Reference test	Lab working times for analysis	Unit of analysis (metric)	Contaminants included?
Schreiber *et al.* (2013)[118]	LightCycler SeptiFast Test - MGRADE not reported Sample: Whole blood Site: NR Volume: NR When samples drawn NR SepsiTest Sample: Whole blood Site: NR Volume: NR When samples drawn NR	Unclear	Immediately after sampling, the containers for blood cultures were transported to the Institute of Medical Microbiology for cultivation at 37°C. The EDTA blood samples were stored at 4–10°C and the NA was extracted within 24 h, according to the manufacturer s' specifications	blood culture and clinical/laboratory confirmation Sample: Whole blood Site: NR Volume: NR blood culture was performed using the BACTEC system	The blood culture and PCR results were compared to the final clinical diagnosis and categorized as true- or false-positive and true- or false-negative.	NR	Patients	Included

Appendix 5: Diagnostic test accuracy, additional information

Table 59: Deviance information criterion (DIC) for SeptiFast compared with blood culture. Standard model (without covariate adjustment) and meta-regression models (with covariates indicating subgroups).

Model		DIC*
Covariate adjustment	**Standard model**	630.10
	Age categories	624.55
	Febrile neutropenia	630.78
	Clinical setting	630.26
	Inclusion/exclusion of contaminants	631.43

*Note that lower values of DIC are favourable, suggesting a more parsimonious model.

Table 60: Coefficient estimates for meta-regression model adjusting for the proportion of patients receiving antibiotics prior to blood draw.

model parameter	regression coefficient, median (95% CrI), logit scale
sensitivity	-0.17(-1.16, 0.78)
specificity	-0.58(-1.24, 0.10)

Note that the regression terms are considered to significantly affect sensitivity and/or specificity if the credible intervals exclude zero (on the logit scale).

Appendix 6: Literature search strategies for the review of cost-effectiveness – A MEDLINE example

Database searched:	Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R)
Platform or provider used:	Ovid SP
Date of coverage:	1948 to May 2015
Search undertaken:	Initial search February 2015
Updated search:	May 2015

1. exp Sepsis/
2. sepsis.mp.
3. septic?emia.mp.
4. Shock, Septic/
5. ((septic or endotoxic or toxic) adj shock).tw.
6. Bacteremia/
7. bacter?emia.mp.
8. Fungemia/
9. fung?emia.mp.
10. Systemic Inflammatory Response Syndrome/
11. sirs.mp.
12. blood$ infection$.tw.
13. blood poison$.tw.
14. or/1-13
15. septifast.mp.
16. lightcycler.mp.
17. 15 or 16
18. 14 and 17
19. sepsitest.mp.
20. iridica.mp.
21. (plex id or plex-id).mp.
22. or/19-21
23. exp Polymerase Chain Reaction/
24. polymerase chain reaction$.tw.
25. pcr$.mp.
26. Gene Amplification/
27. Nucleic Acid Amplification Techniques/
28. or/23-27
29. Genes, Bacterial/ or Genes, Fungal/
30. (exp bacteria/ or exp Fungi/) and exp Nucleic Acids/
31. ((bacteri$ or fung$) adj3 (dna or gene$ or nucleic acid$)).tw.
32. blood culture$.tw.
33. or/29-32
34. 14 and 28 and 33
35. 18 or 22 or 34
36. Animals/ not (Humans/ and Animals/)
37. 35 not 36
38. limit 37 to yr="2006 -Current"
39. exp "Costs and Cost Analysis"/
40. Economics/ (26570)
41. exp Economics, Hospital/
42. exp Economics, Medical/

43 Economics, Nursing/
44 exp models, economic/
45 Economics, Pharmaceutical/
46 exp "Fees and Charges"/
47 exp Budgets/
48 budget$.tw.
49 ec.fs.
50 cost$.ti.
51 (cost$ adj2 (effective$ or utilit$ or benefit$ or minimi$)).ab.
52 (economic$ or pharmacoeconomic$ or pharmaco-economic$).ti.
53 (price$ or pricing$).tw.
54 (financial or finance or finances or financed).tw.
55 (fee or fees).tw.
56 (value adj2 (money or monetary)).tw.
57 quality-adjusted life years/
58 (qaly or qalys).af.
59 (quality adjusted life year or quality adjusted life years).af.
60 or/39-59
61 38 and 60
62 38 not 61

Appendix 7: Population of key parameters by clinical estimates- reproduction of the correspondence sent to the clinical experts

The task for the clinical expert is to provide a midpoint estimate together with a range for the variables shown in Tables 1 and 2. We would like this estimate provided in terms of a single positive test result. Tables 1 and 2 differ in that the Table 1assumes that the results from standard blood culture process are concordant with the positive test result, whereas Table 2 assumes that the results from the standard blood culture process are negative. It is acknowledged that blood culture results would not be known when the result from the rapid test becomes available, but it was believed that formulating the question in this manner would make the task easier for the clinician, and these data can be weighted by rates of true positives and false positives by the researchers.

Illustrative examples are provided. For example, If you believed that the information provided by a positive SeptiFast result would produce a net average reduction in ICU length of stay of 0.1 days compared with not having the information from SeptiFast then -0.1 would be entered into the top left cell. Were it believed that a positive MALDI-TOF MS test would be associated with a net average reduction of 0.001 in 30-day mortality then -0.001 would be entered into the bottom right cell. If it is believed that the answers differ for subgroups, such as children and neonates, people who are immunocompromised, those with recent antibiotic use, and people with suspected health care acquired infection and suspected community acquired infection, then please duplicate the tables with appropriate data.

In order to aid clinical judgement data that may be considered useful is contained following Table 2 although the generalisability of the data to treatment in England in 2015 needs to be assessed. These data have been split into two categories, data obtained from systematic reviews, and additional data. The data from the systematic reviews were identified either through the review of diagnostic accuracy undertaken by ScHARR or by a review undertaken by the NICE Guideline Development Group when constructing the draft guidelines on antimicrobial stewardship.

The additional data has been sourced from studies identified within the cost effectiveness searches undertaken by ScHARR. These were supplemented by citation searching. As such, the results cannot be classed as derived from a systematic review.

Table 1: Template to be completed by the clinical expert. Assuming that the result from the blood culture process is positive and in agreement with the test

	LightCycler SeptiFast Test MGRADE	SepsiTest	IRIDICA BAC BSI	MALDI-TOF MS
Average net effect on ICU length of stay				
Average net effect on hospital length of stay				
Average net effect on the cost of antimicrobials				
Net effect on 30-day mortality				

Table 2: Template to be completed by the clinical expert. Assuming that the result from the blood culture process is negative

	LightCycler SeptiFast Test MGRADE	SepsiTest	IRIDICA BAC BSI
Average net effect on ICU length of stay			
Average net effect on hospital length of stay			
Average net effect on the cost of antimicrobials			
Net effect on 30-day mortality			

Information that may be considered useful:

Data from systematic reviews

- From an RCT[1] the mean time to SeptiFast result was 15.9 hours compared with 38.1 hours for blood culture plus MALDI-TOF MS. No data from RCTs on the timings of a result being known were available for SepsiTest or IRIDICA BAC BSI. The same RCT[1] reports the mean time spent in ICU as 34 days for the SeptiFast and 32 days for blood culture plus MALDI-TOF MS. This was not statistically significant.

- An RCT[2] of de-escalation of antimicrobials recruiting 116 patients with severe sepsis reported statistically significantly greater rates of superinfection in the de-escalation group (27% vs 11%; p-value = 0.03) and in the mean number of antibiotic days (9 vs 7.5; p-value = 0.03). There was a non-statistically significant increase in median duration of ICU stay (9 days vs 8 days; p-value = 0.71) in the de-escalation arm

Additional data

- A paper[3] reports the implementation of an evidence-based intervention that integrated MALDI-TOF MS, rapid antimicrobial susceptibility testing, and near–real-time antimicrobial stewardship practices. Comparison of results before and after were made. The mean hospital

length of stay after blood stream infection onset in the pre-intervention group survivors (n = 100) was 9.9 versus 8.1 days in the intervention group (n=101; p-value=.01). Within a multivariate model receiving active antibiotic therapy at 48 hours was associated with a hazard ratio for discharge of 2.90 (95% CI 1.15-7.33; p-value = 0.02) and the intervention was associated with a hazard ratio for discharge of (95% CI 1.01-1.88; p-value = 0.04). Total hospitalisation costs was $45,709 in the pre-intervention cohort vs $26,162 in the intervention.

- A further paper reporting a pre–post quasi-experimental study analysed the impact of MALDI-TOF MS with an antimicrobial stewardship team.[4] The intervention (n = 256) decreased time to organism identification (84.0 vs 55.9 hours, p-value < .001), and improved time to effective antibiotic therapy (30.1 vs 20.4 hours, p-value = .021), optimal antibiotic therapy (90.3 vs 47.3 hours, p-value < .001) and length of ICU stay (14.9 vs 8.3 days, p-value = .014) compared with pre-intervention (n=245). 30-day all-cause mortality was lower in the intervention arm compared with pre-intervention (12.73 vs 20.3%. p-value = .021) as was length of hospitalisation (14.2 vs 11.4 days, p-value = .066)

- An Italian observational, propensity matched analysis[5] comparing a retrospective cohort with a prospective cohort (using SeptiFast) in haematological patients – typically acute myeloid leukaemia. Propensity matching was undertaken for: definitive blood culture; positive blood cultures; negative blood cultures; (and patients with positive SeptiFast and patients with negative SeptiFast results. No differences were observed in the length of stay or in changes in management. The mortality difference in the original propensity score matching was not significant 8.24 vs 13.48 p = 0.39). However, in a more stringently matched group SeptiFast was reported to have better mortality rates (3.13% compared with 14.71% p-value =0.04). There were lower costs (€431; p-value = 0.05) in the prospective cohort compared with the retrospective cohort.

- One study[6] aimed to evaluate the economic impact of SeptiFast via a cost-minimisation study. 48 patients were in the SeptiFast group with 54 in control. The paper concluded that there was a 94.6% chance of cost savings associated with use of SeptiFast when samples were run per batch. A large proportion of these savings were from reduced ICU length of stay although this could be heavily confounded by the demographic and clinical data of the SeptiFast and control groups. For example, there were 20 patients with heart surgery in the control and 2 in the SeptiFast group, and 4 polytrauma / head injuries in the control group compared with 20 in the SeptiFast group.

- A prospective, observational trial in 2 German university hospitals, 1 Spanish, 1 American and 1 Italian tertiary hospital compared the use of SeptiFast with Blood Culture.[7] This study estimated that if SeptiFast had been used then there would have been 22.8 days reduction in inadequate treatment per 100 tests. The results for those in ICU alone were taken and it was estimated that the SeptiFast could have presented 5 mortalities from 221 investigated sepsis episodes within 30 days of discontinuing antimicrobial treatment.[8]. However, the data relating inadequate treatment to mortality were taken from studies published in 2000 or earlier.[9,10]

- A study in Texas compared the outcomes of 112 patients with antibiotic-resistant Gram-negative bacteraemia, during January 2009 – November 2011 with 157 patients during February 2012 to June 2013 post intervention following the introduction of an intervention (MALDI-TOF MS and antimicrobial stewardship).[11] Time to initiation of active treatment was 90 hours pre-intervention and 32 hours post intervention ($p<0.001$). There were 33 (21%) and 10 (9%) all-cause mortalities observed in the pre-intervention cohort and the intervention cohort respectively. In multivariate logistic regression the intervention was a significant predictor of survival (OR=0.28, 0.12-0.71; p-value =0.008). A significant reduction in average total hospital costs was observed from $78,991 to $52,693.

- A paper by Martiny *et al*.,[12] reports that the use of MALDI-TOF MS resulted in the modification of in treatment in 21/157 adults and 1/40 paediatrics

- A Spanish retrospective matched cohort study[13] attempted to determine the attributable mortality and excess length of stay associated with inadequate empirical antimicrobial therapy between 1997 – 2006. Therapy was considered inadequate when no effective drug against the isolated pathogen(s) was included in the empirical antibiotic treatment within the first 24 hours of admission to the ICU, or the doses and pattern of administration were not in accordance with current medical standards. From 87 matched pairs 59 (67.8%) died in the inadequate group compared with 25 (28.7%) in the control group. Removing pairs with nosocomial infection still showed a 31.4% excess in mortality (65.7% vs 34.3%). In those without a nosocomial infection there was a significant reduction in the length of stay in ICU associated with adequate treatment (7 vs 9 days; p-value = 0.02)

- Using a generalised linear model, adjusted for confounders, Zilberberg *et al*.,[14] estimated that the excess length of hospitalisation was 7.7 days (95% CI 0.6-13.5) and attributable costs were $13,398 (95% CI $1,060-$26,736) when a patient had inadequate antifungal treatment. Inadequate antifungal treatment was defined as treatment delay of ≥24 hours from Candidemia onset or inadequate dose of antifungal agent active against the pathogen.

- Arnold *et al*.,[15] attempted to estimate from 167 consecutive patients the costs of inappropriate treatment of Candidemia, which was defined as delayed antifungal therapy >24 hours from culture collection. 22 patients had appropriate therapy, 145 did not. Length of stay was shorter in the appropriately treated group (7 vs 10.4 days; p-value = 0.037) and the costs were lower ($15,832 vs $33,021; p-value <0.001)

- Morrell *et al*.,[16] retrospectively analysed 157 consecutive patients over a 4-year period with a candida bloodstream infection of which 50 (32%) died during hospitalisation. The number of people without a delay in antifungal treatment (>12 hours) was 9, whilst 148 patients had delayed treatment. Adjusted odds ratio associated with delay in antifungal treatment was 2.09 (95% CI 1.53-2.84). Delay in antifungal treatment was also associated with a longer duration within ICU (9.4 days vs 0.4 days; p-value = 0.019).

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Appendix 8: Results from the threshold analyses

The threshold analyses are divided into three categories based on the number of samples assumed to require analysing per day (2.4, 17 or 68). In each category each intervention is compared both with blood culture and with MALDI-TOF MS. In these analyses it is assumed that the comparator has already been purchased and that the intervention will require purchasing.

Assuming 2.4 samples a day require analysing.

Threshold analyses for SeptiFast versus blood culture

Figure 24: Threshold analyses for SeptiFast versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 2.4 samples analysed per day

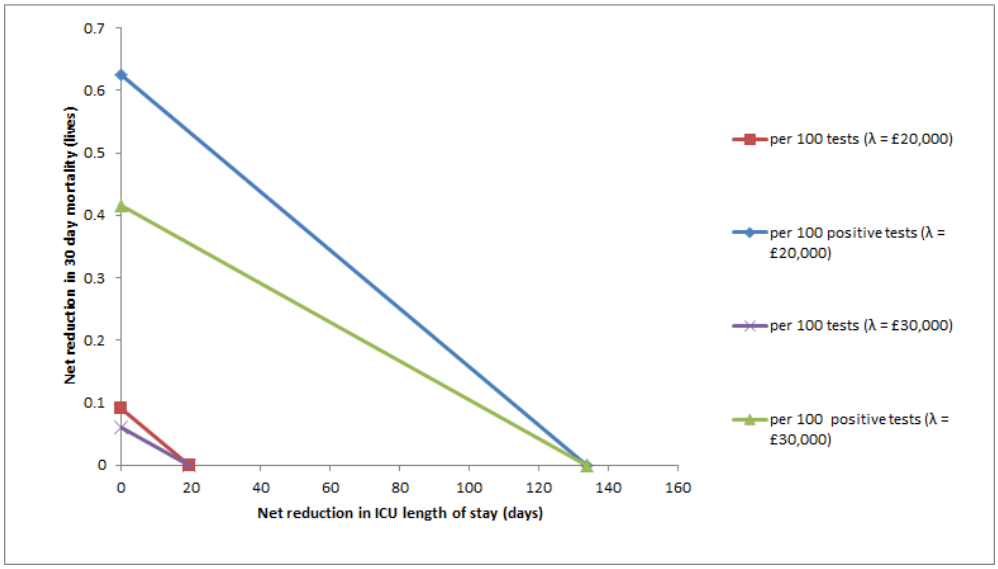
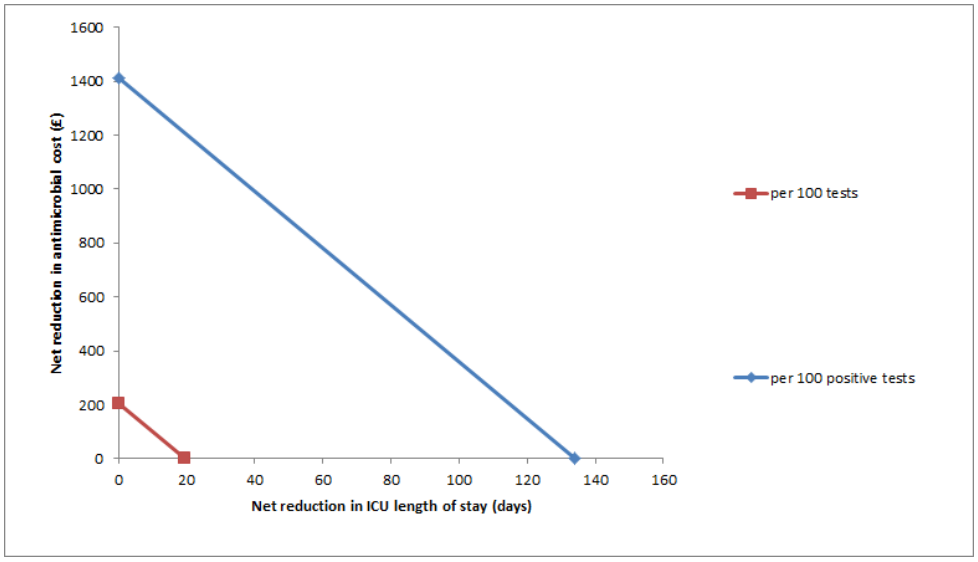


Figure 25: Threshold analyses for SeptiFast versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 2.4 samples analysed per day



Threshold analyses for SeptiFast versus MALDI-TOF MS

Figure 26: Threshold analyses for SeptiFast versus MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 2.4 samples analysed per day

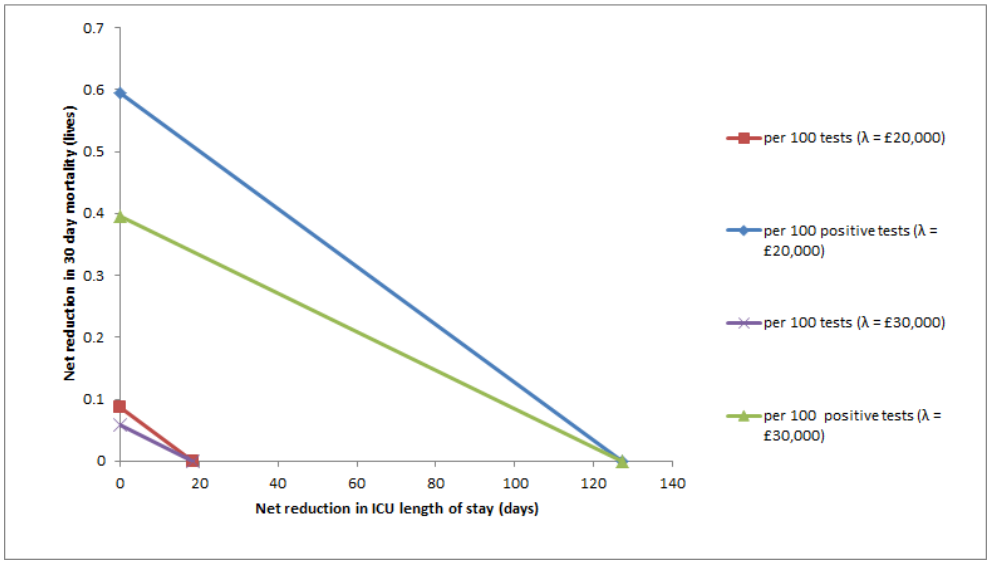
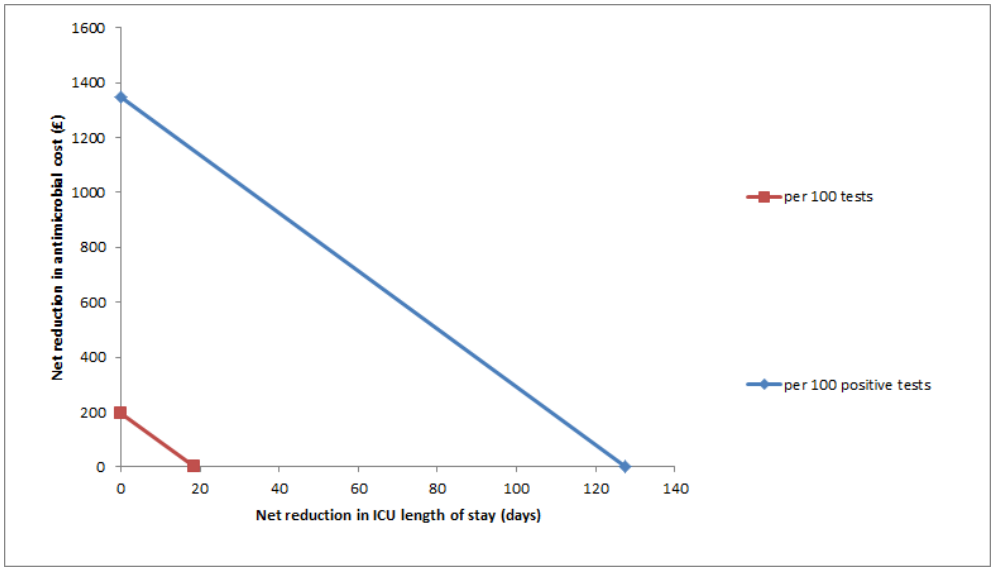


Figure 27: Threshold analyses for SeptiFast versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 2.4 samples analysed per day



Threshold analyses for SepsiTest versus blood culture

Figure 28: Threshold analyses for SepsiTest versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 2.4 samples analysed per day

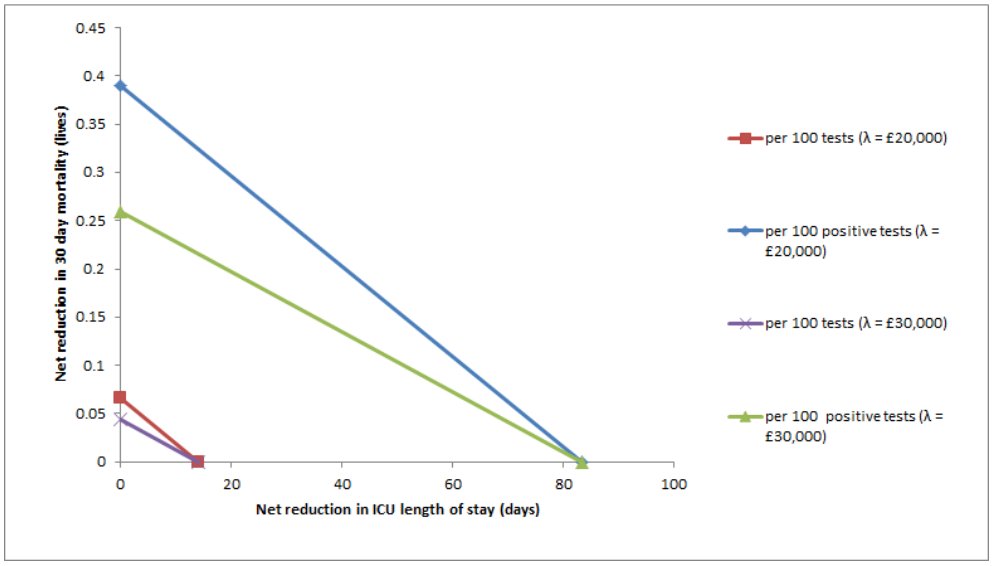
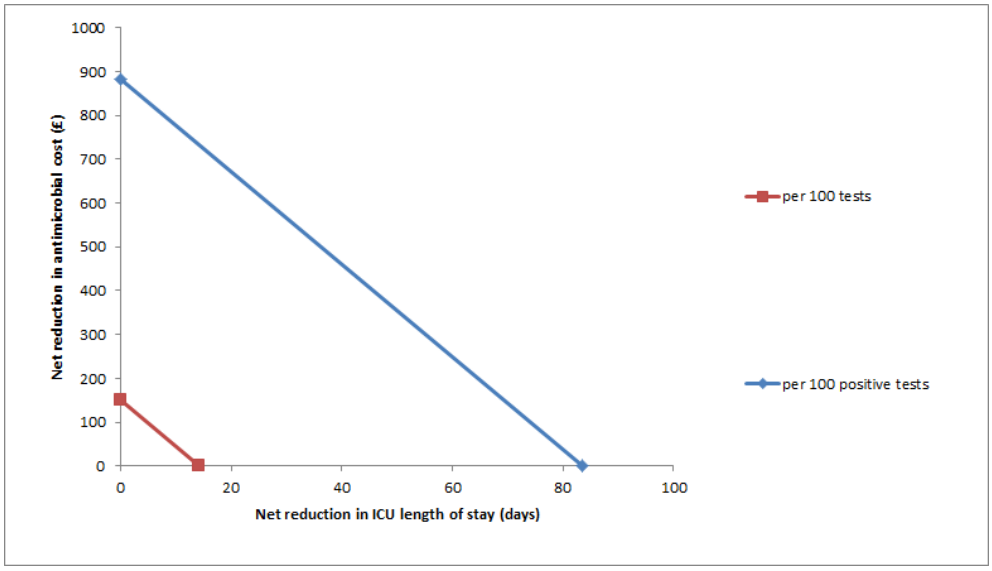


Figure 29: Threshold analyses for SepsiTest versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 2.4 samples analysed per day



Threshold analyses for SeptiTest versus MALDI-TOF MS

Figure 30: Threshold analyses for SeptiTest versus MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the machinery related to SepsiTest needs to be purchased. Assuming 2.4 samples analysed per day

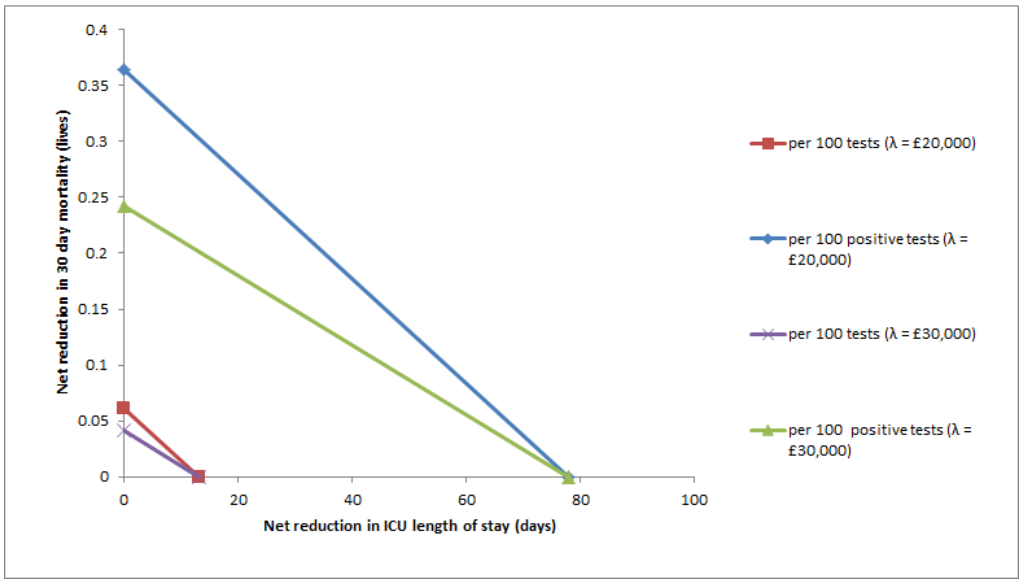
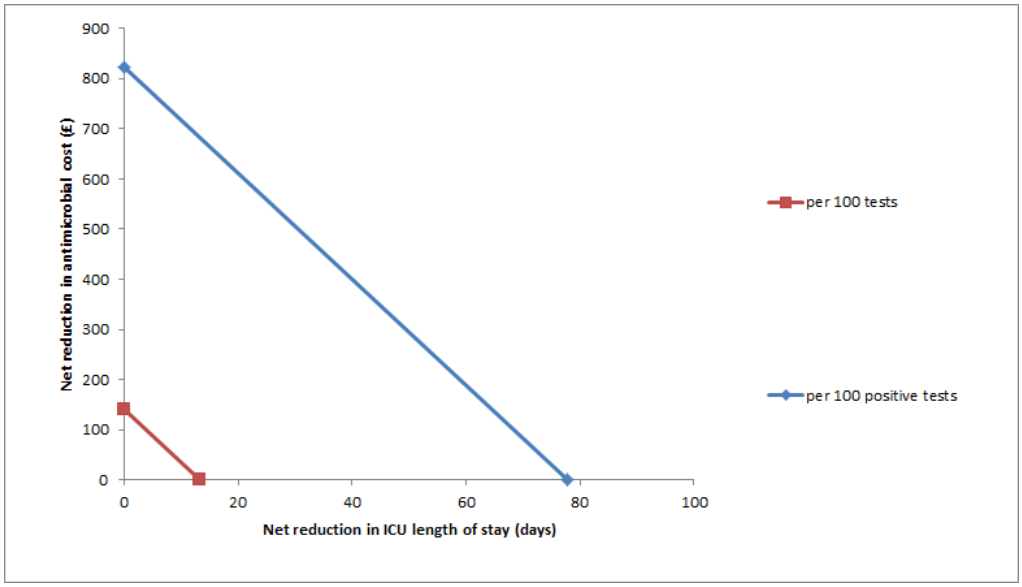


Figure 31: Threshold analyses for SepsiTest versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 2.4 samples analysed per day



Threshold analyses for IRIDICA versus blood culture

Figure 32: Threshold analyses for IRIDICA versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 2.4 samples analysed per day

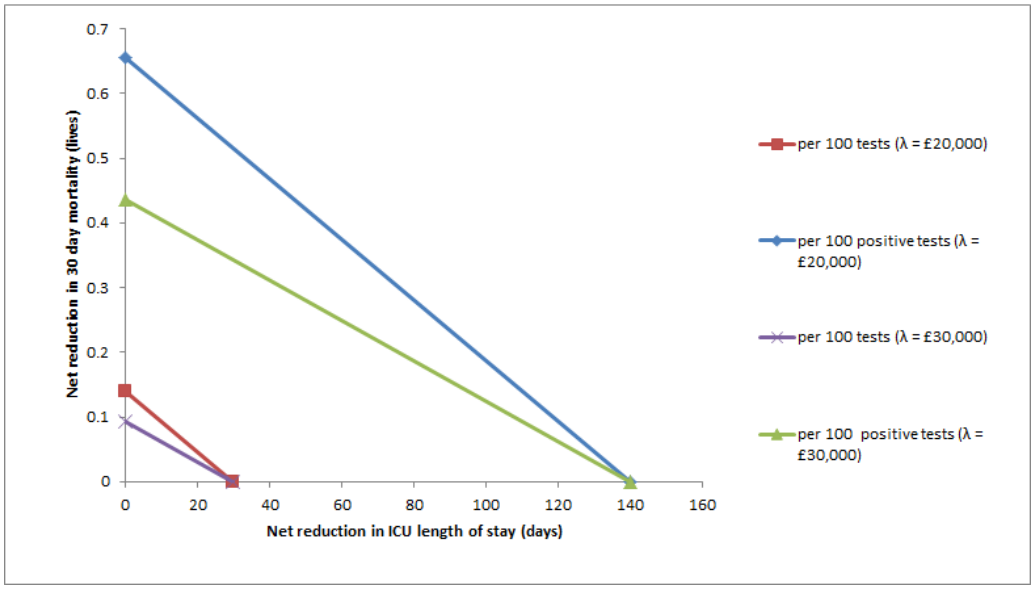
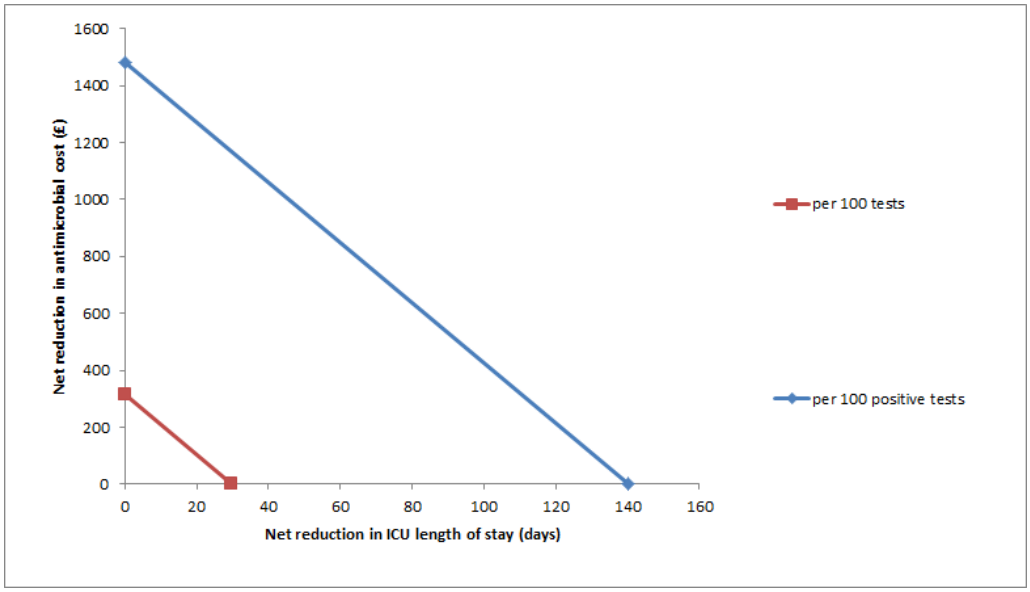


Figure 33: Threshold analyses for IRIDICA versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 2.4 samples analysed per day



Threshold analyses for IRIDICA versus MALDI-TOF MS

Figure 34: Threshold analyses for IRIDICA versus MALDI_TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 2.4 samples analysed per day

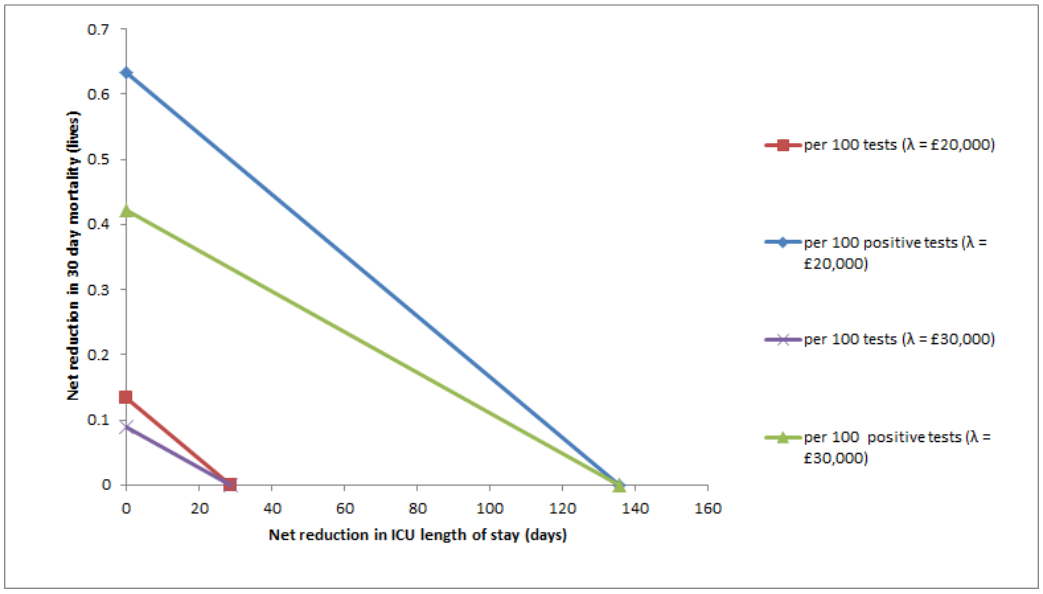
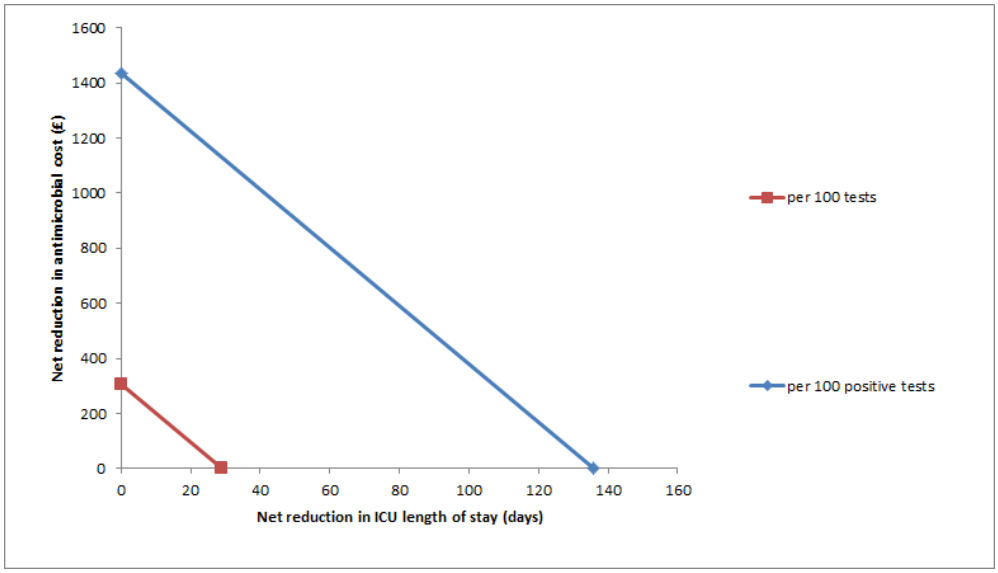


Figure 35: Threshold analyses for IRIDICA versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 2.4 samples analysed per day



Assuming 17 samples a day require analysing.

Threshold analyses for SeptiFast versus blood culture

Figure 36: Threshold analyses for SeptiFast versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 17 samples analysed per day

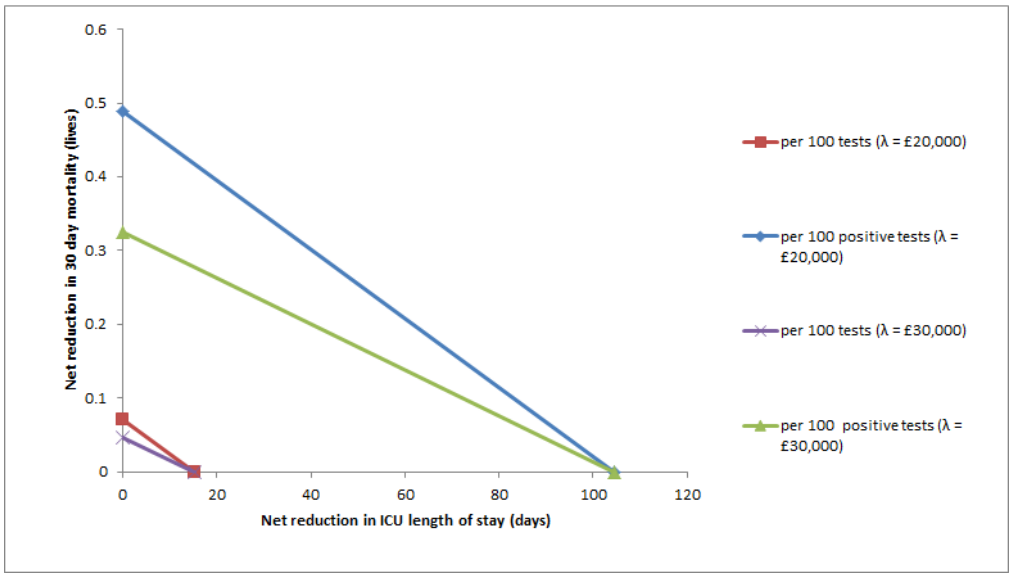
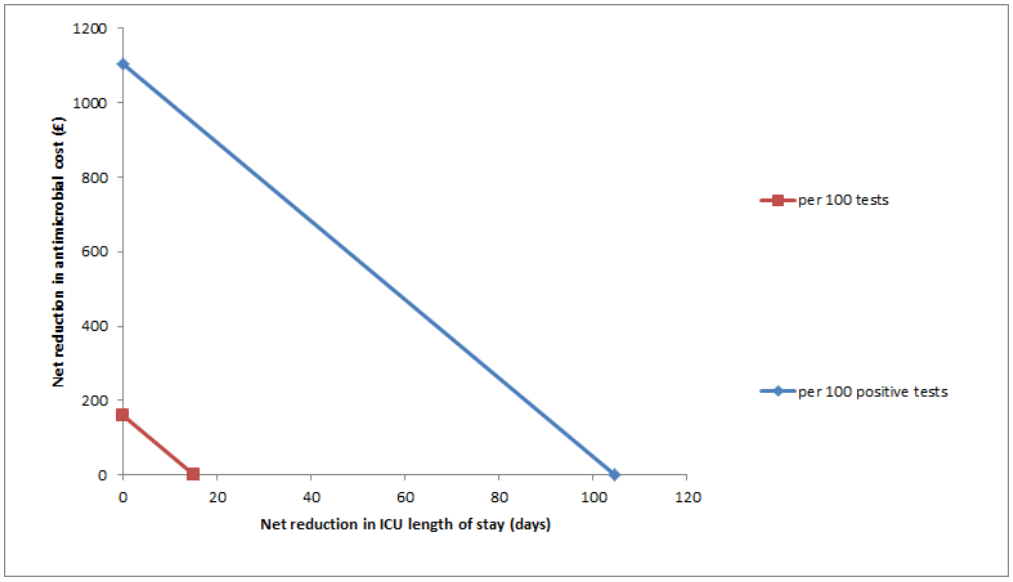


Figure 37: Threshold analyses for SeptiFast versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 17 samples analysed per day



Threshold analyses for SeptiFast versus MALDI-TOF MS

Figure 38: Threshold analyses for SeptiFast versus MALDI_TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 17 samples analysed per day

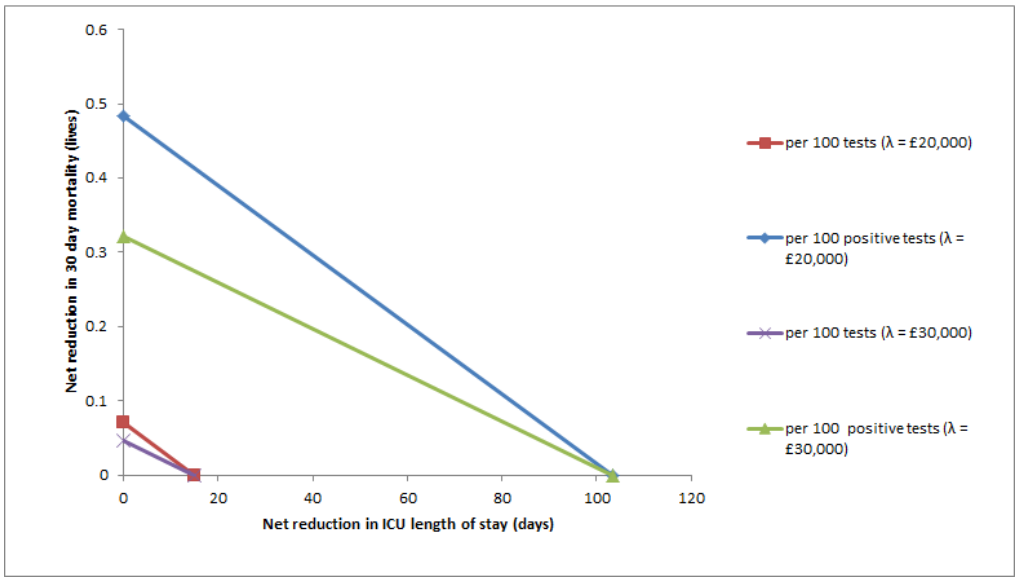
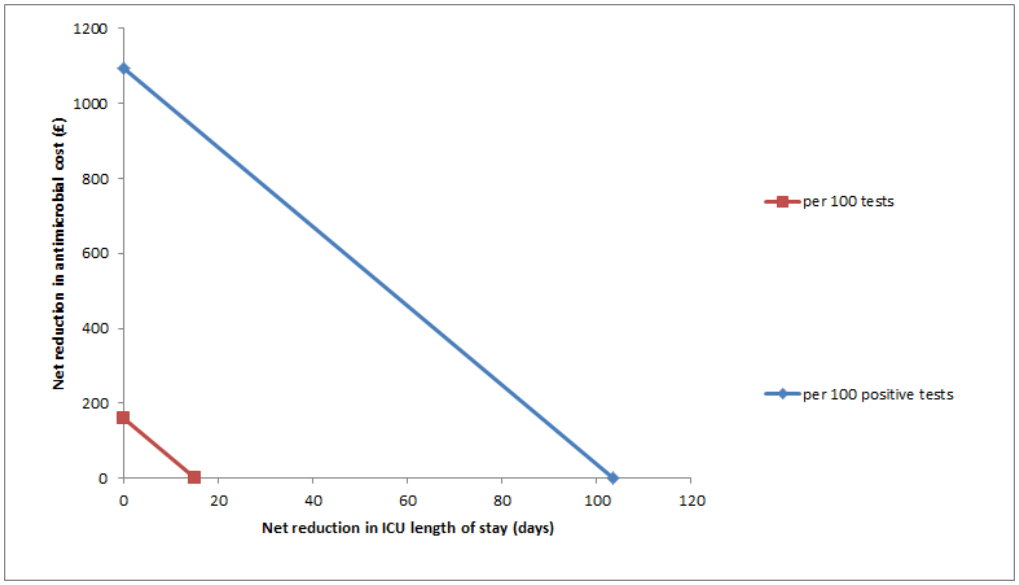


Figure 39: Threshold analyses for SeptiFast versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 17 samples analysed per day



Threshold analyses for SepsiTest versus blood culture

Figure 40: Threshold analyses for SepsiTest versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 17 samples analysed per day

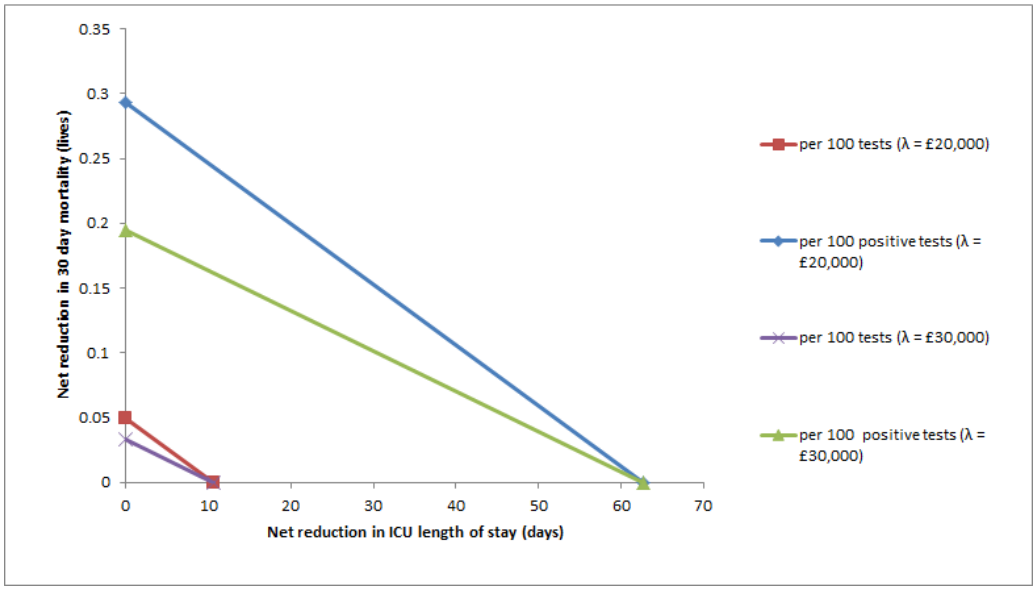
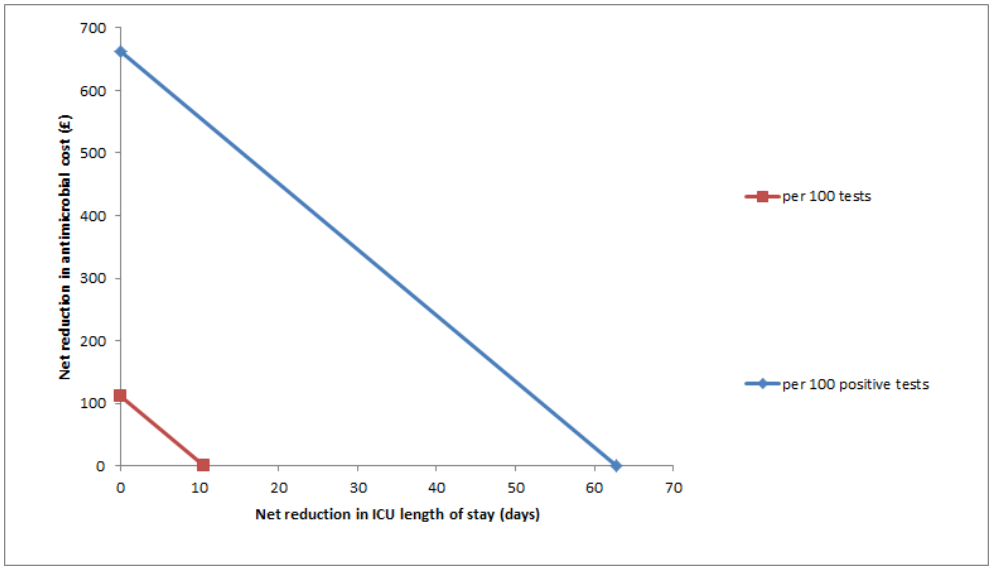


Figure 41: Threshold analyses for SepsiTest versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 17 samples analysed per day



Threshold analyses for SeptiTest versus MALDI-TOF MS

Figure 42: Threshold analyses for SeptiTest versus MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the machinery related to SepsiTest needs to be purchased. Assuming 17 samples analysed per day

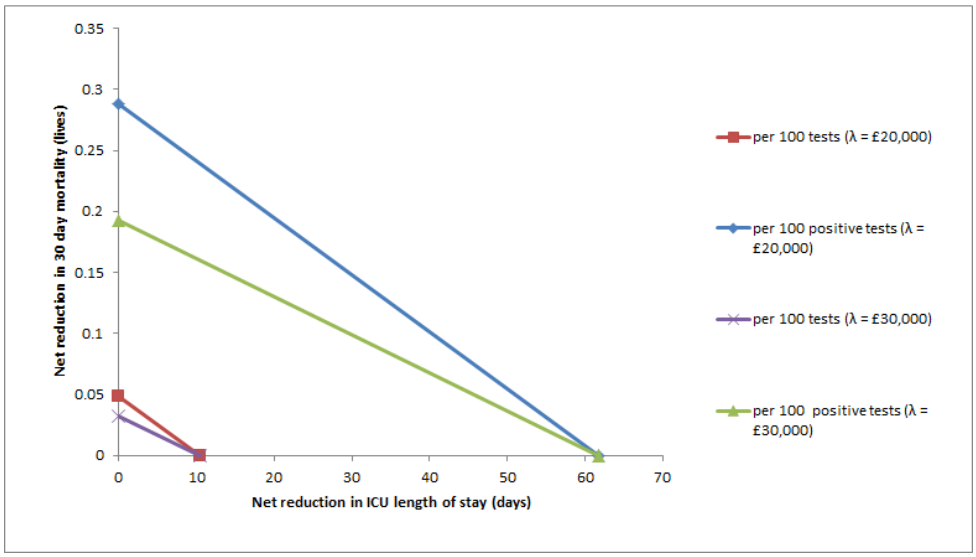
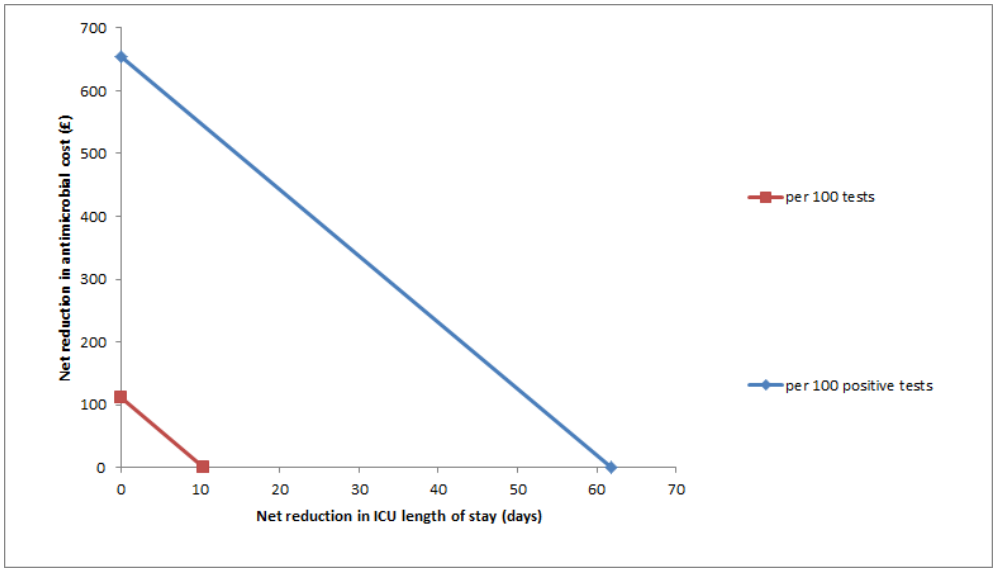


Figure 43: Threshold analyses for SepsiTest versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 17 samples analysed per day



Threshold analyses for IRIDICA versus blood culture

Figure 44: Threshold analyses for IRIDICA versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 17 samples analysed per day

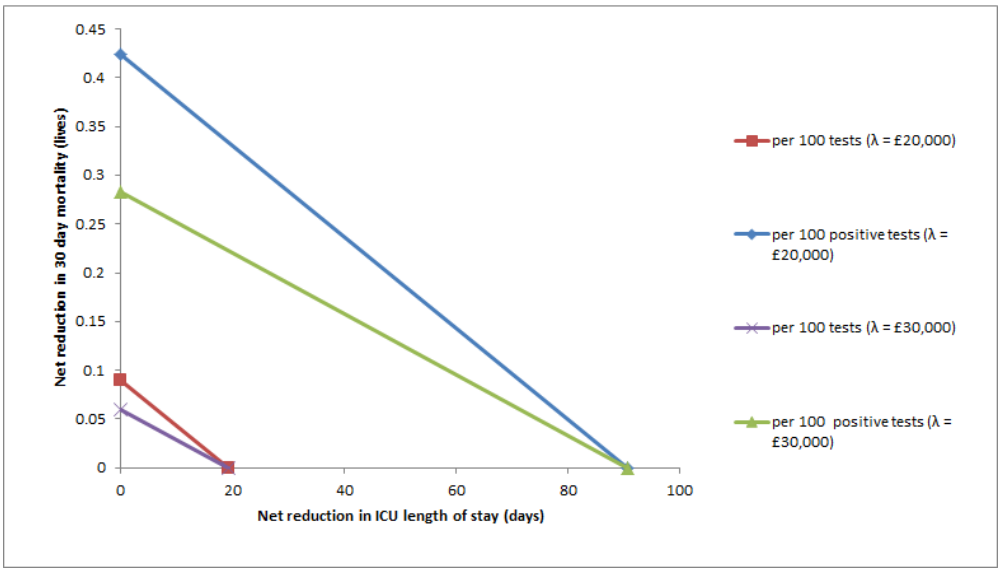
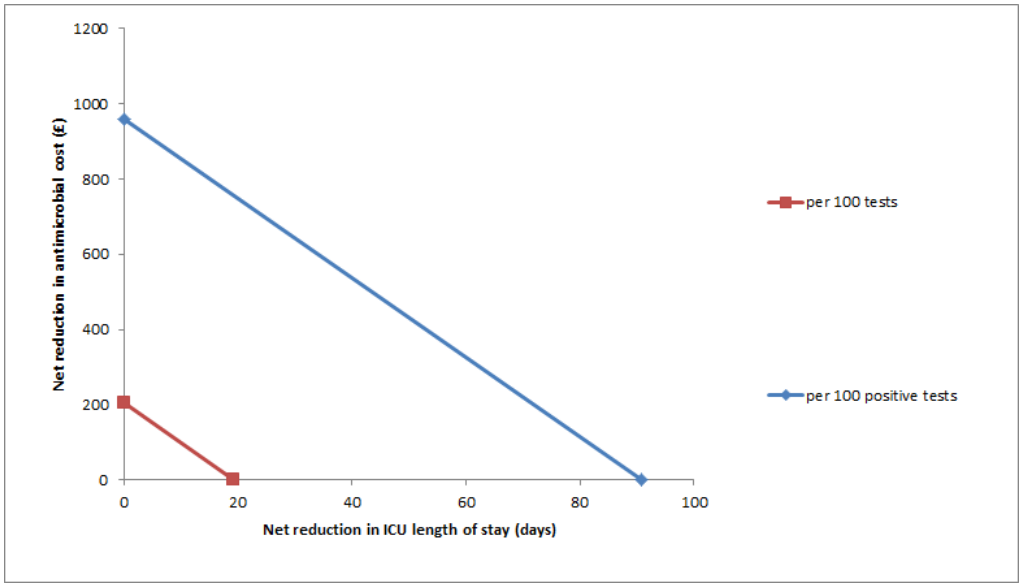


Figure 45: Threshold analyses for IRIDICA versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 17 samples analysed per day



Threshold analyses for IRIDICA versus MALDI-TOF MS

Figure 46: Threshold analyses for IRIDICA versus MALDI_TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased . Assuming 17 samples analysed per day

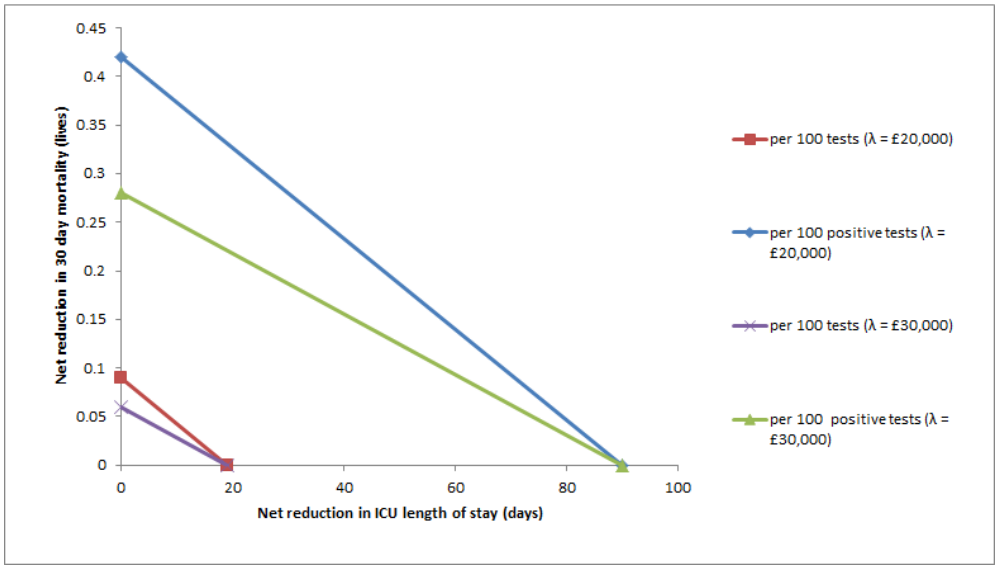
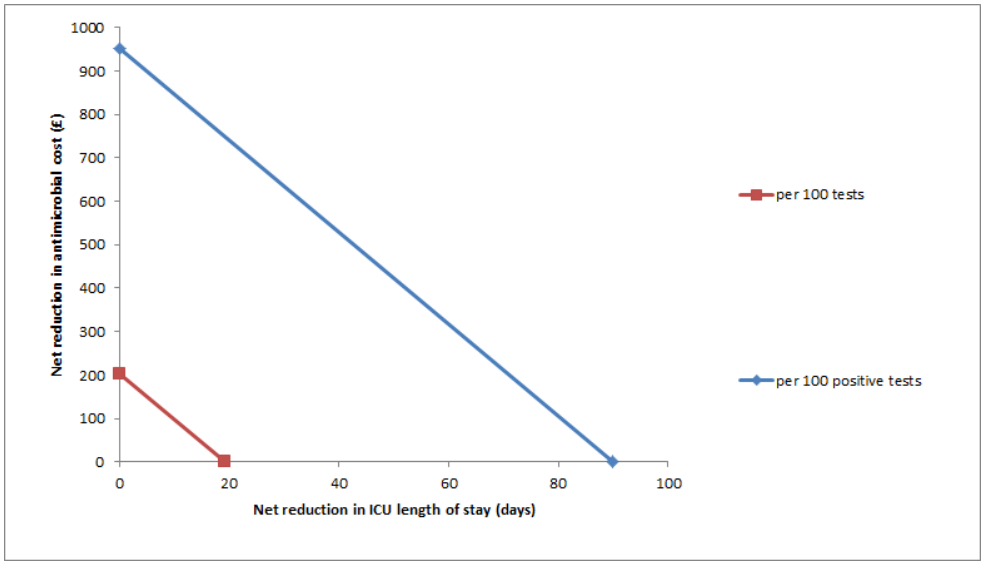


Figure 47: Threshold analyses for IRIDICA versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 17 samples analysed per day



Assuming 68 samples a day require analysing.

Threshold analyses for SeptiFast versus blood culture

Figure 48: Threshold analyses for SeptiFast versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 68 samples analysed per day

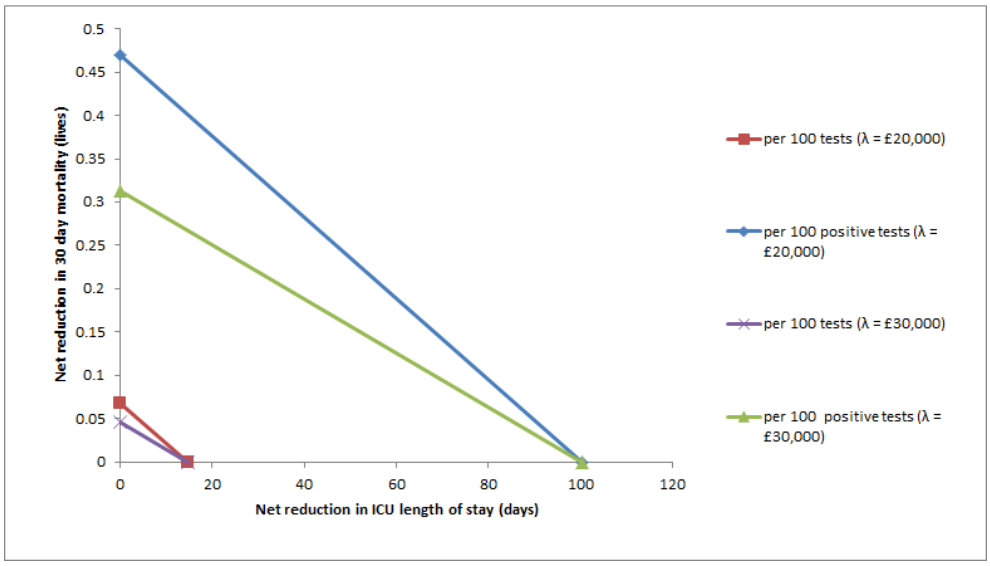
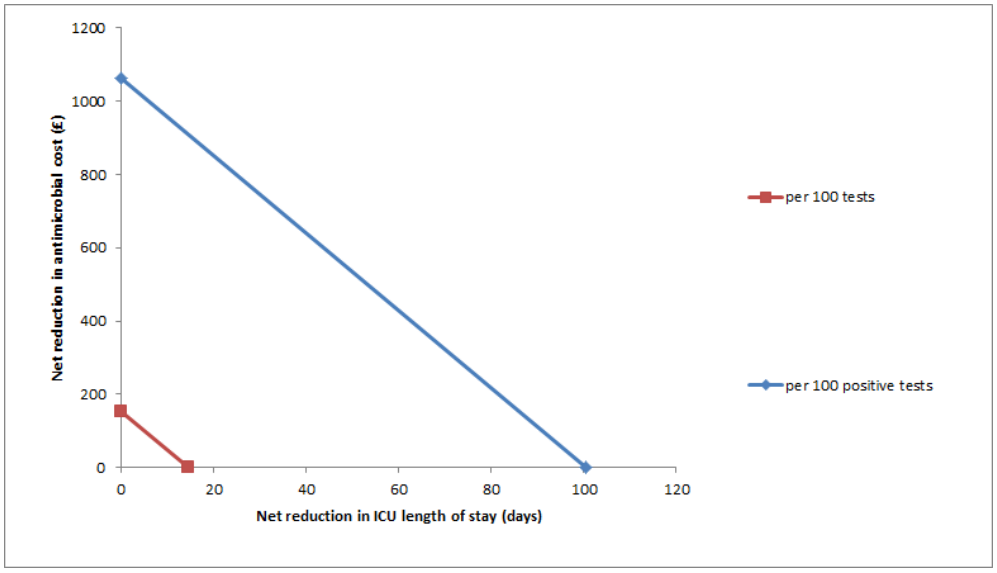


Figure 49: Threshold analyses for SeptiFast versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 68 samples analysed per day



Threshold analyses for SeptiFast versus MALDI-TOF MS

Figure 50: Threshold analyses for SeptiFast versus MALDI_TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 68 samples analysed per day

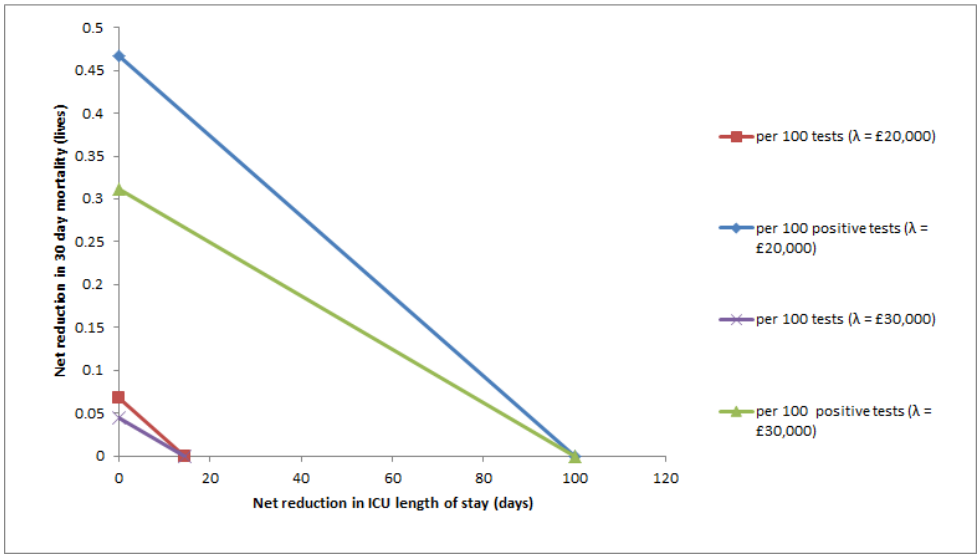
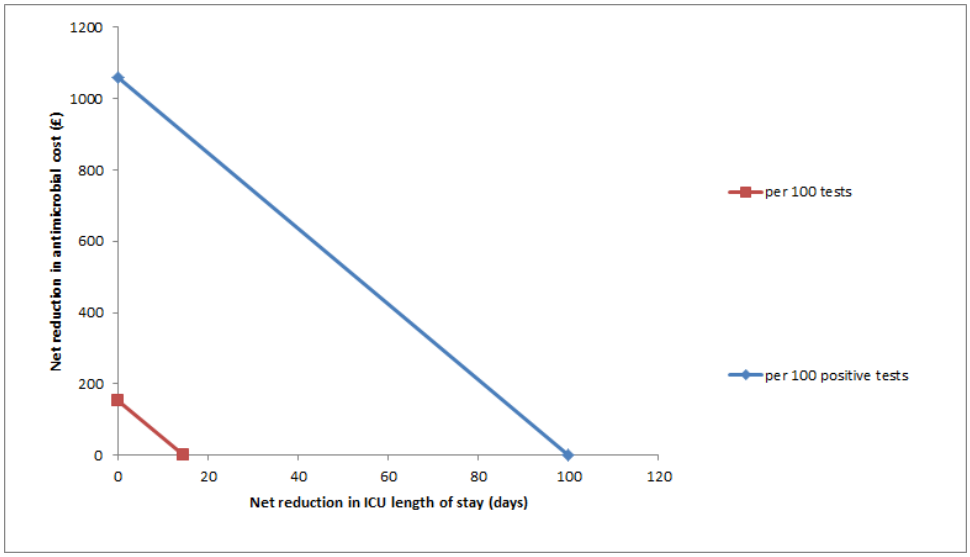


Figure 51: Threshold analyses for SeptiFast versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the SeptiFast machine needs to be purchased. Assuming 68 samples analysed per day



Threshold analyses for SepsiTest versus blood culture

Figure 52: Threshold analyses for SepsiTest versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 68 samples analysed per day

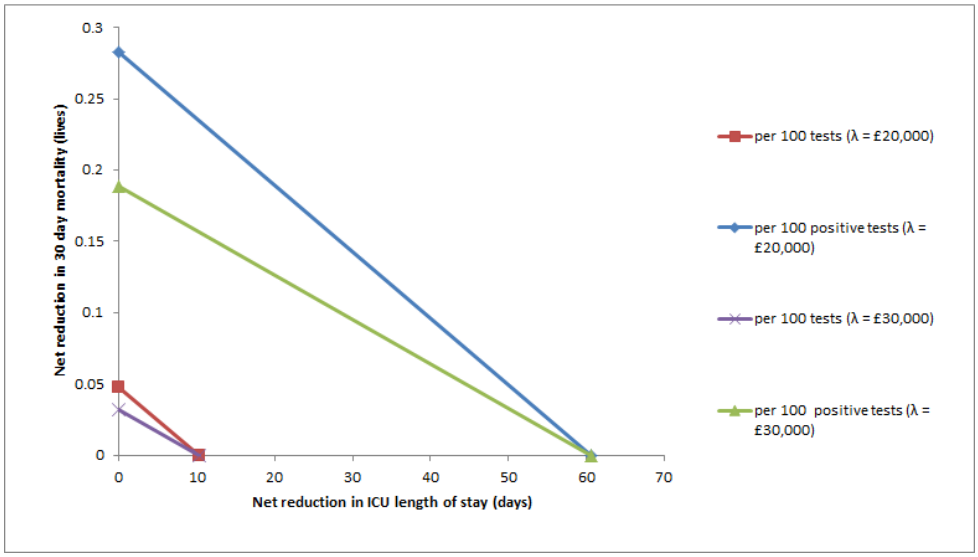
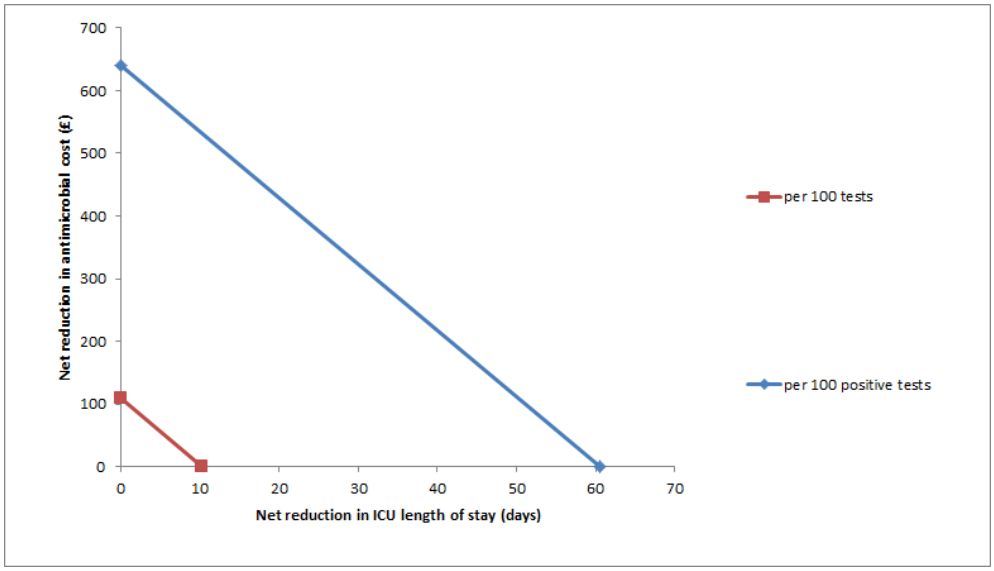


Figure 53: Threshold analyses for SepsiTest versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 68 samples analysed per day



Threshold analyses for SeptiTest versus MALDI-TOF MS

Figure 54: Threshold analyses for SeptiTest versus MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the machinery related to SepsiTest needs to be purchased. Assuming 68 samples analysed per day

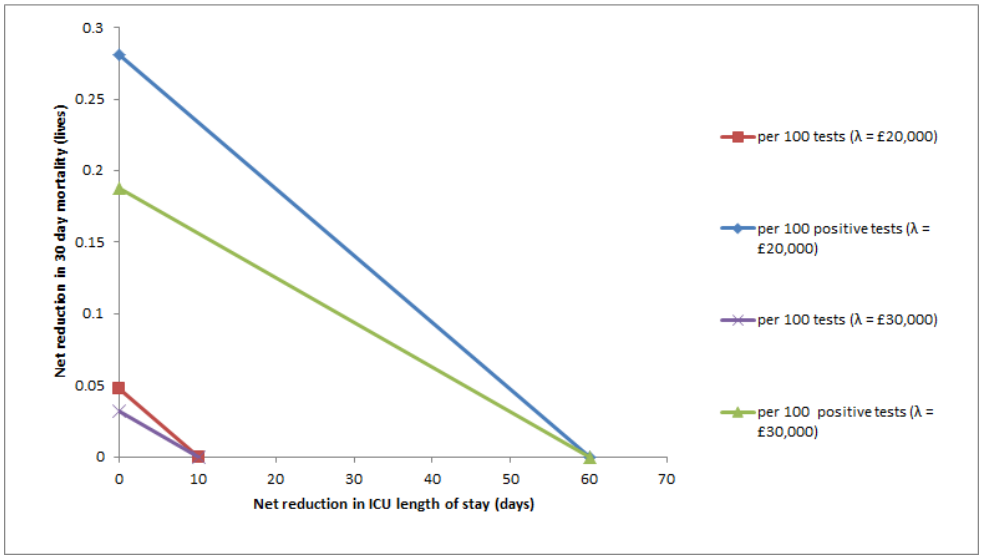
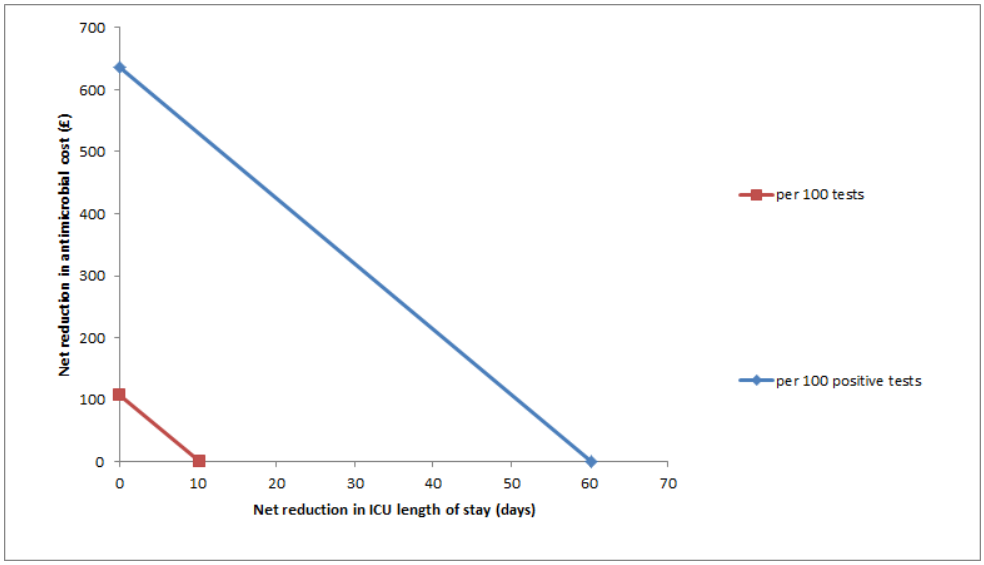


Figure 55: Threshold analyses for SepsiTest versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming machinery related to SepsiTest needs to be purchased. Assuming 68 samples analysed per day



Threshold analyses for IRIDICA versus blood culture

Figure 56: Threshold analyses for IRIDICA versus blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 68 samples analysed per day

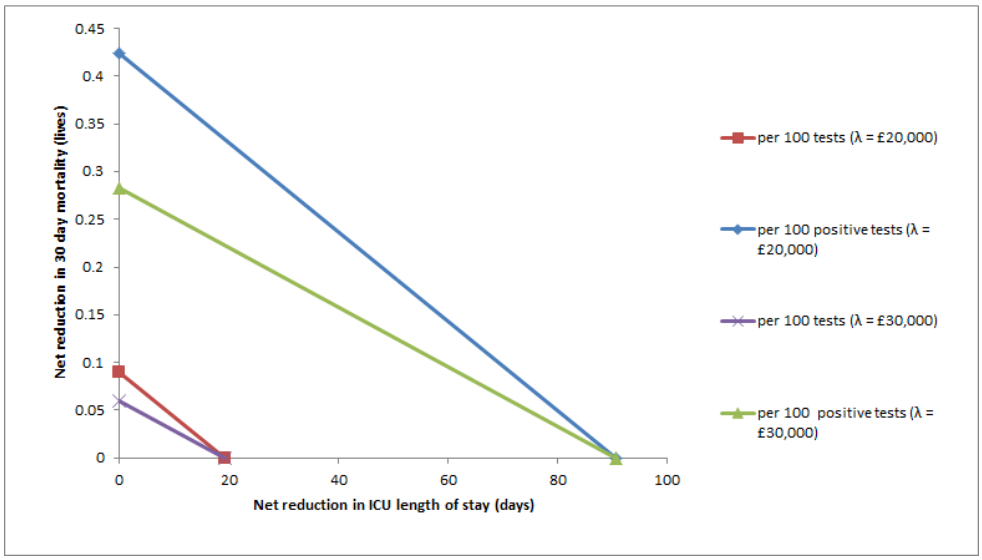
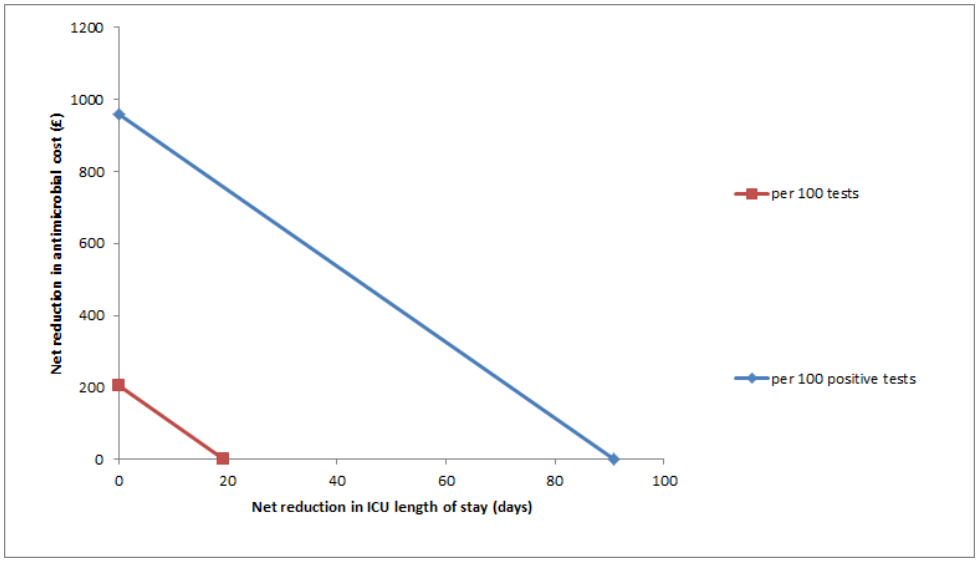


Figure 57: Threshold analyses for IRIDICA versus blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 68 samples analysed per day



Threshold analyses for IRIDICA versus MALDI-TOF MS

Figure 58: Threshold analyses for IRIDICA versus MALDI_TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased . Assuming 68 samples analysed per day

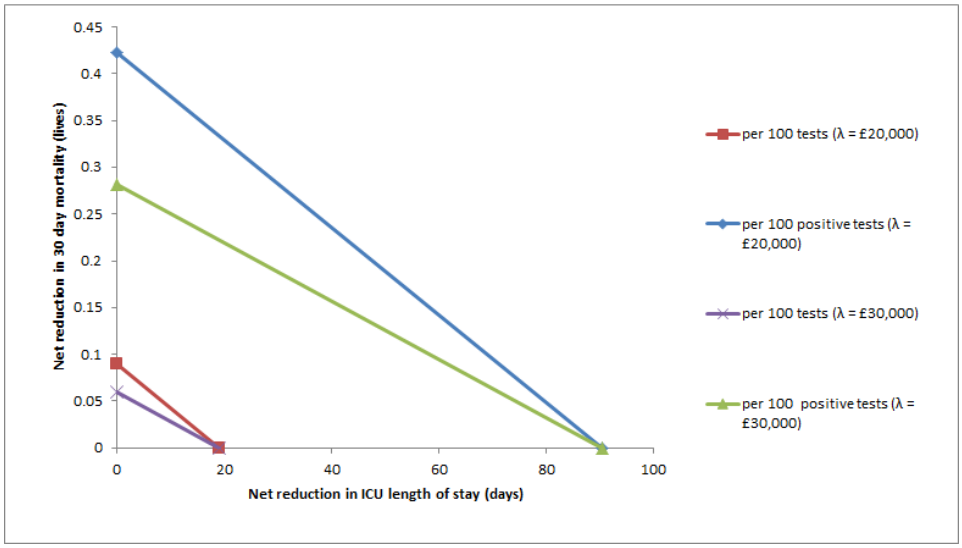
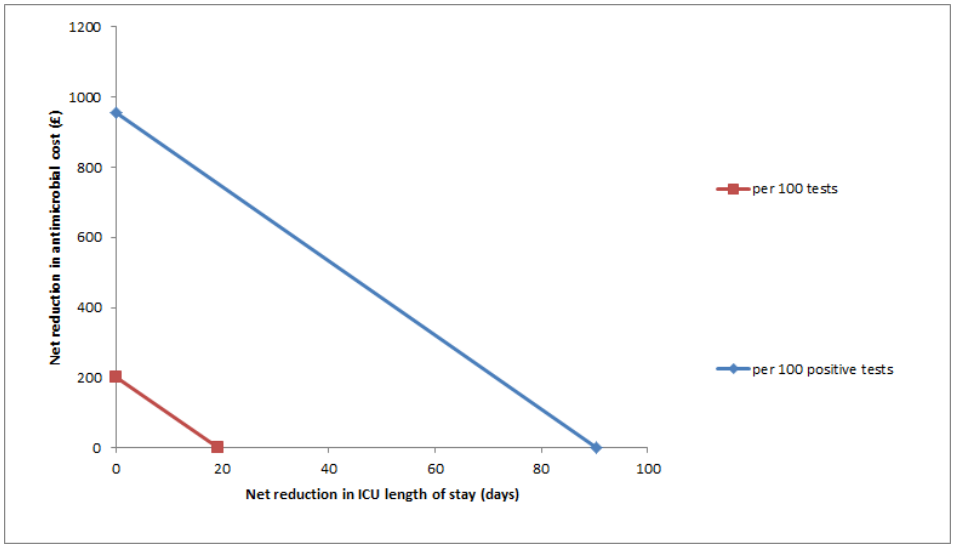


Figure 59: Threshold analyses for IRIDICA versus MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming the IRIDICA machine needs to be purchased. Assuming 68 samples analysed per day



National Institute for Health and Care Excellence

DIAGNOSTICS ASSESSMENT PROGRAMME

Evidence overview

Sepsis: The LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi

This overview summarises the key issues for the Diagnostics Advisory Committee's consideration. This document is intended to be read in conjunction with the final scope issued by NICE for the assessment and the diagnostics assessment report. A glossary of terms can be found in Appendix B.

1 Background

1.1 Introduction

The purpose of this assessment is to evaluate the clinical and cost effectiveness of the LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assays for rapidly identifying bacterial and fungal DNA, which may be present in the bloodstream of people who are suspected of having sepsis. The assays are molecular in-vitro diagnostic tests which are intended to be used in conjunction with clinical assessment. Whole blood samples can be used and no prior-incubation or pre-culture steps are required. Current microbiology techniques require blood samples to be incubated and cultured before the identification of viable pathogens so the ability to directly test whole blood samples could result in pathogens being identified earlier and enable prompt medical intervention. This may be of particular benefit in people who are suspected of having a severe infection. The use of the assays may also reduce the length of use of broad spectrum

antibiotics and antifungals and facilitate targeted treatment earlier in the care pathway. It is anticipated that blood culture would still be required to provide definitive antimicrobial susceptibility data, where this is not provided by the rapid diagnostic test.

Provisional recommendations on the use of these technologies will be formulated by the Diagnostics Advisory Committee at the Committee meeting on 1 September 2015.

1.2 Scope of the evaluation

Table 1 scope of the evaluation

Decision question	What is the clinical and cost-effectiveness of using the LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay in addition to clinical assessment for rapidly identifying bloodstream bacteria and fungi?
Populations	People with suspected bloodstream infections in secondary care Potential subgroups include: • People with a suspected healthcare associated infection • People with a suspected community acquired infection • Children and neonates • People who are immunocompromised • People exposed to antibiotics prior to blood sample collection
Interventions	• LightCycler SeptiFast Test MGRADE • SepsiTest • IRIDICA BAC BSI assay in conjunction with clinical assessment
Comparator	• Clinical assessment in conjunction with blood culture • Clinical assessment in conjunction with blood culture and MALDI-TOF
Healthcare setting	• Departments and wards providing care for acutely unwell patients

	• Critical care unit
Outcomes	Intermediate measures for consideration may include: • Diagnostic accuracy • Discordant results with blood culture • Time to result • Time to treatment decision • Test failure rates • Duration of ICU and/or hospital stay • Duration of broad and narrow spectrum antimicrobial therapy • Re-admission rate • Change in antimicrobial treatment plan
	Clinical outcomes for consideration may include: • Side-effects associated with broad spectrum antimicrobial use • Morbidity and mortality • Severity of disease (as measured by scoring systems such as SOFA, SAPS II and APACHEII) • Rates of superinfection (including *C. difficile*) • Rates of resistant infections • Health related quality of life
	Costs will be considered from an NHS and Personal Social Services perspective. Costs for consideration may include: • Cost of equipment, reagents and consumables • Cost of staff and associated training • Costs associated with treatment (for example, broad and narrow spectrum antibiotics and antifungals) • Medical costs arising from testing and care such as hospital stay • Medical costs arising from adverse events including those associated with false test results and inappropriate treatment Blood culture in current practice is required for the identification of bloodstream bacteria and fungi, and to

	provide definitive antimicrobial susceptibility data. It is anticipated that blood cultures would be required in addition to the rapid molecular tests, to provide definitive antimicrobial susceptibility data.
	The cost-effectiveness of interventions should be expressed in terms of incremental cost per quality-adjusted life year.
	The potential costs and health impacts associated with antimicrobial resistance should also be considered.
Time horizon	The time horizon for estimating clinical and cost effectiveness should be sufficiently long to reflect any differences in costs or outcomes between the technologies being compared.

Further details including descriptions of the interventions, comparators, care pathway and outcomes can be found in the final scope.

2 The evidence

This section summarises data from the diagnostics assessment report compiled by the External Assessment Group.

2.1 Clinical Effectiveness

The External Assessment Group conducted a systematic review of the evidence on the clinical effectiveness of the LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay. Details of the systematic review can be found starting on page 23 of the diagnostics assessment report.

Studies were included if they evaluated one of the interventions compared with either blood culture or blood culture with MALDI-TOF mass spectrometry (MS) on blood samples collected from patients during the management of suspected sepsis. Studies which compared 1 of the interventions with another were also included. In total, 66 studies met the inclusion criteria; 62 studies reported diagnostic accuracy data and were included in meta-analyses, and 41 studies reported intermediate or clinical outcome measures and were

included in a narrative analysis. Meta-analyses of diagnostic accuracy data were based on a bivariate normal model with Markov Chain Monte Carlo simulation. Inter-study heterogeneity was explored using meta-regression. Details of the meta-analyses methods can be found starting on page 27 of the diagnostics assessment report.

Sixty four of the 66 studies were single index test single-gate studies, that is studies in which only patients with the target condition (suspected sepsis) are recruited. Three of these studies were randomised controlled trials (RCT). The remaining 2 studies were single gate studies which reported results for both the LightCycler SeptiFast Test MGRADE and SepsiTest. Only 3 of the 66 studies included patients from the UK, with the majority of studies conducted in other European countries. Of the studies that included patients from the UK, one study (Dark et al. 2009) used the SeptiFast assay to assess 50 patients and one other study (Vincent et al. in press) used the IRIDICA assay to assess [redacted] patients from 6 European countries including the UK. The third UK study (Warhurst et al. 2015) reported the use of SeptiFast in 795 patients with sepsis and was judged to be the highest quality and most applicable included study.

All studies were assessed using the QUADAS-2 tool. 65 studies were considered to be at risk of bias and had concerns regarding applicability. The issues of greatest concern included patient selection and blinding to the index test or reference standard. The External Assessment Group also reported concerns about 21 studies which did not report whether the blood samples for the index test and reference standard were drawn at the same time, and 6 studies which used a mixture of reference standards. In addition, only 28 studies reported that the blood sampling and test methods used were in accordance with the company's instructions for use. The unit of analysis also differed between studies with diagnostic accuracy data reported for patients, sample episodes, or species and pathogen level.

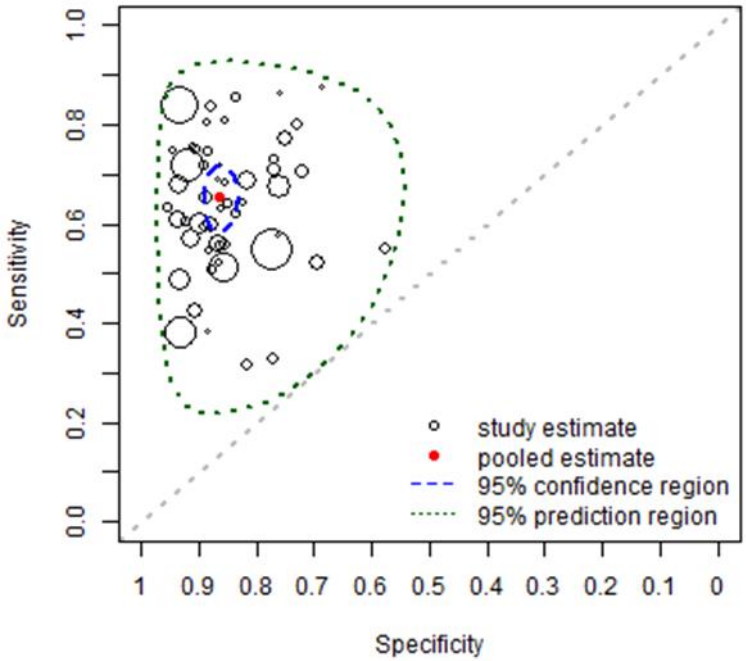
Diagnostic accuracy

Of the 62 studies that reported diagnostic accuracy data, 55 reported data for the LightCycler SeptiFast Test MGRADE, 5 reported data for SepsiTest and 4 reported data for the IRIDICA BAC BSI assay. Two of the 62 studies reported data for both the Light Cycler SeptiFast Test MGRADE and SepsiTest and were counted as individual studies for each test.

LightCycler SeptiFast Test MGRADE

54 studies compared the LightCycler SeptiFast Test MGRADE with blood culture and were combined in a meta-analysis. The pooled estimate for sensitivity was 0.65 (95% credible interval 0.60 to 0.71; 95% prediction interval 0.29 to 0.90) and for specificity was 0.86 (95% credible interval 0.84 to 0.89; 95% prediction interval 0.62 to 0.96). The summary ROC curve for this analysis is reproduced below in figure 1. The proportion of discordant results varied across studies from 6% to 46% (median 17%).

Figure 1 summary ROC curve LightCycler SeptiFast Test MGRADE compared with blood culture (54 studies)



One study (Tafelski et al. 2015) compared the LightCycler SeptiFast Test MGRADE with blood culture plus MALDI-TOF MS. It reported a sensitivity of 0.58 (95% confidence interval 0.30 to 0.86) and a specificity of 0.74 (95% confidence interval 0.64 to 0.85). Further details of these analyses can be found starting on page 57 of the diagnostics assessment report.

Subgroup analyses

Reasons for heterogeneity in sensitivity and specificity estimates between studies were explored using meta-regression for clinically relevant variables. The following variables were explored:

- Age (neonates and children)
- Exposure to antibiotics prior to blood sample collection
- Suspected community or health care acquired infection
- Febrile neutropenia
- Studies with inclusion/exclusion of contaminants

There was no evidence that sensitivity and specificity estimates were affected by these variables.

SepsiTest

Four studies compared SepsiTest with blood culture and were combined in a meta-analysis. The pooled estimate for sensitivity was 0.48 (95% credible interval 0.21 to 0.74; 95% prediction interval 0.07 to 0.90) and for specificity was 0.86 (95% credible interval 0.78 to 0.92; 95% prediction interval 0.66 to 0.95). The summary ROC curve for this analysis is reproduced below in figure 2. The proportion of discordant results varied between studies and ranged from 14% to 26% (median 22%).

Figure 2 [redacted]

One study (Loonen et al. 2014) compared SepsiTest with blood culture plus MALDI-TOF MS. The study reported a sensitivity of 0.11 (95% confidence interval 0.00 to 0.23) and specificity of 0.96 (95% confidence interval 0.92 to 1.00). No subgroup analyses were possible for the SepsiTest. Further details of these analyses can be found on pages 61 to 63 of the diagnostics assessment report.

IRIDICA BAC BSI

Four studies compared the IRIDICA BAC BSI assay with blood culture and were combined in a meta-analysis. Two of these studies reported data using a previous version of the IRIDICA PCR/ESI-MS analyser known as the PLEX-ID which has different desalter and mass spectrometry modules. The pooled estimate for sensitivity was 0.81 (95% credible interval 0.69 to 0.90; 95% prediction interval 0.55 to 0.94) and for specificity was 0.84 (95% credible interval 0.71 to 0.92; 95% prediction interval 0.50 to 0.96). The summary ROC curve for this analysis is reproduced below in figure 3. The proportion of discordant results varied between studies and ranged from 7% to 30% (median 18%).

Figure 3 [redacted]

No studies compared the IRIDICA BAC BSI assay with blood culture plus MALDI-TOF MS and no subgroup analyses were possible for this intervention. Further details of this analysis can be found on pages 63 to 64 of the diagnostics assessment report.

Intermediate and clinical outcomes

Forty one studies were included which reported data related to the time to pathogen identification for the index test, time to treatment, test failure rate, mortality, duration of ICU and/or hospital stay, duration of antibiotic therapy or reported changes in antimicrobial treatment plan. None of the included studies reported data on re-admission rates, adverse events associated with broad spectrum antimicrobial use, morbidity, changes in disease severity over time, rates of superinfection, rates of resistant infection or health related quality of life.

LightCycler SeptiFast Test MGRADE

Thirty seven studies reported data on intermediate and clinical outcomes for the LightCycler SeptiFast Test MGRADE. In addition one study (Schreiber et al. 2013) reported data for both the LightCycler SeptiFast Test MGRADE and SepsiTest. No studies compared the LightCycler SeptiFast Test MGRADE with the IRIDICA BAC BSI assay. Full details of these analyses can be found starting on page 64 of the diagnostics assessment report. The results of the narrative analysis are summarised below.

Time to result (pathogen identification)

Twenty one studies reported the time to pathogen identification, which reported turnaround times from a minimum of 4 hours to a median of 26.25 hours. Some studies reported the time for pathogen identification using blood cultures which reported a minimum of 24 hours and a median of 80 hours.

Time to treatment adaptation

Three RCTs reported time to treatment. Tafelski et al. (2015) reported a mean time from taking the blood sample to adapting therapy of 18.8 hours (SD 5.6) for the LightCycler SeptiFast Test MGRADE and 38.3 hours (SD 14.5) for blood culture and MALDI-TOF MS. 9.8% of patients had therapy changed after a positive LightCycler SeptiFast Test MGRADE result compared with 13.5% in the blood culture and MALDI-TOF MS group. Rodrigues et al. (2015)

reported a mean time to change of therapy of 9.7 hours with the LightCycler SeptiFast Test MGRADE compared with 50.1 hours for blood culture (p=0.004). 3.5% of patients in the LightCycler SeptiFast Test MGRADE had therapy adjusted compared with 24% in the blood culture group. Idelevich et al. (2015) reported a mean time to adapting treatment of 21.4 hours (range 16.2 to 46.3 hours) in the LightCycler SeptiFast Test MGRADE group compared with 47.5 hours (range 7.3 to 59.2 hours) in the blood culture group (p=0.018).

Test failure rates

Seven studies reported test failure rates which ranged from 1.5% to 24.2%. It is not clear why there is a large variation in failure rates between studies.

Length of intensive care unit and/or hospital stay

Thirteen studies which compared the LightCycler SeptiFast Test MGRADE with blood culture reported length of intensive care unit, hospital stay or both. In the majority of the studies reporting these data it was often unclear if the length of stay was up to, including or after blood sampling. Also, the majority of studies did not present comparative data. One study (Alvarez et al. 2012) reported a statistically significant difference ($p<0.05$) in intensive care unit and hospital length of stay in favour of the LightCycler SeptiFast Test MGRADE and three studies (Idelevich et al. 2014, Mancini et al. 2014 and Rodrigues et al. 2013) reported no significant difference in length of stay.

Duration of broad and narrow spectrum antibiotic therapy

One RCT (Tafelski et al. 2015) reported a duration of antimicrobial therapy of 18.8 hours (±5.6 SD) for the LightCycler SeptiFast Test MGRADE compared with 38.3 hours (±14.5 SD) for blood culture.

Change in antimicrobial treatment plan

Fourteen studies reported details of change in antimicrobial treatment planning, 10 of which did not report comparative data. One RCT (Rodrigues et al. 2013) reported that 35% of patients in the LightCycler SeptiFast Test

MGRADE group had an adjustment to therapy compared with 21% of patients in the blood culture group. In contrast, a further RCT (Idelevich et al. 2015) reported that 9.5% of patients in the LightCycler SeptiFast Test MGRADE had an adjustment to therapy compared with 10.5% in the blood culture group. One study based on propensity score matching (Mancini et al. 2014) reported no differences in management.

One RCT (Tafelski et al. 2015) compared the LightCycler SeptiFast Test MGRADE with blood culture plus MALDI-TOF MS and reported that 9.8% of patients had their therapy changed as a result of testing with the LightCycler SeptiFast Test MGRADE compared with 13.5% for blood culture plus MALDI-TOF MS.

Mortality

Seventeen studies reported mortality data and 12 of these studies reported data on a cohort level only. The mortality rates reported ranged from 4% to 61%; however, the length of follow-up was highly variable across the studies. One study (Alvarez et al. 2012) reported no statistically significant differences between the LightCycler SeptiFast Test MGRADE and blood culture for both 28 day and 6 months mortality. A further study (Rodrigues et al. 2013) also reported no statistically significant difference in 28 day mortality.

One propensity score matching study (Mancini et al. 2014) reported no statistically significant difference in mortality (p=0.39) between a prospective cohort (LightCycler SeptiFast Test MGRADE) and retrospective cohort (blood culture), although when more stringent matching criteria were applied the LightCycler SeptiFast Test MGRADE was associated with a statistically significant reduction in mortality (3.13% compared with 14.71%; p=0.04). A reduction in mortality associated with using the LightCycler SeptiFast Test MGRADE was reported in 2 further studies (Idelevich et al. 2015 and Tafelski et al. 2015) but the reductions were not statistically significant.

SepsiTest

Mortality

One study (Loonen et al. 2014) reported a mortality rate of 3.2% for the study cohort but the duration of follow-up was not reported. In addition Schreiber et al. (2013) reported an intensive care unit mortality rate of 16% and a 28 day mortality rate 24% for the study cohort.

No other intermediate or clinical outcome data were reported for the SepsiTest.

IRIDICA BAC BSI

Test failure rates

One study (Metzgar et al. unpublished) reported a rate of test validity of [redacted] which suggests a failure rate of approximately [redacted] for the IRIDICA BAC BSI assay.

Change in antimicrobial treatment plan

One study (Vincent et al. in press) reported that an adjudication panel of 3 clinical experts retrospectively recommended a change in management based on the IRIDICA BAC BSI assay for [redacted] of all patients. This [redacted] of patients in cases where the IRIDICA BAC BSI assay was positive and blood culture was negative.

Mortality

Once study (Vincent et al. in press) reported a mortality rate of [redacted]

2.2 Costs and cost effectiveness

The External Assessment Group conducted a search to identify existing studies investigating the cost effectiveness of the LightCycler SeptiFast Test MGRADE, SepsiTest or the IRIDICA BAC BSI assay. The External

Assessment Group also constructed a de novo mathematical model to determine the cost effectiveness of the technologies.

Systematic review of cost effectiveness evidence

Details of the methods used for the systematic review of the economic evidence can be found starting on page 79 of the diagnostics assessment report. Four studies were included and were assessed according to their relevance to the decision problem: 3 studies included the LightCycler SeptiFast Test MGRADE, 2 of which were within-study cost minimisation analyses and 1 a cost effectiveness analysis; and the remaining study included a cost minimisation analysis of the IRIDICA PLEX-ID hybrid assay. The target population, condition and setting varied between the 4 studies.

The 2 studies which were within-study cost minimisation analyses of using the LightCycler SeptiFast Test MGRADE when compared with blood culture reported cost savings of €178.75 per sample (Mancini et al. 2014) and €183 per patient (Alvarez et al. 2012). The third study, Lehmann et al. (2010), reported ICERs of €11,477 per incremental survivor and €3,107 per QALY gained when using the LightCycler SeptiFast Test MGRADE compared with blood culture. When the use of an IRIDICA-PLEX-ID hybrid test was compared with blood culture, Bilkovski et al. (2014) reported cost savings of $1,123,372 per 422 tests. None of the studies considered the impact of a potential reduction in antibiotic resistance. The External Assessment Group concluded that the existing economic evaluations had limited relevance to either the UK or the decision problem because of differences in patient populations, costs of the interventions and standard care. In particular, Mancini et al. (2014) included haematology patients who were prescribed relatively expensive empirical antifungals that are unlikely to be representative of the UK treatment pathway.

Economic analysis

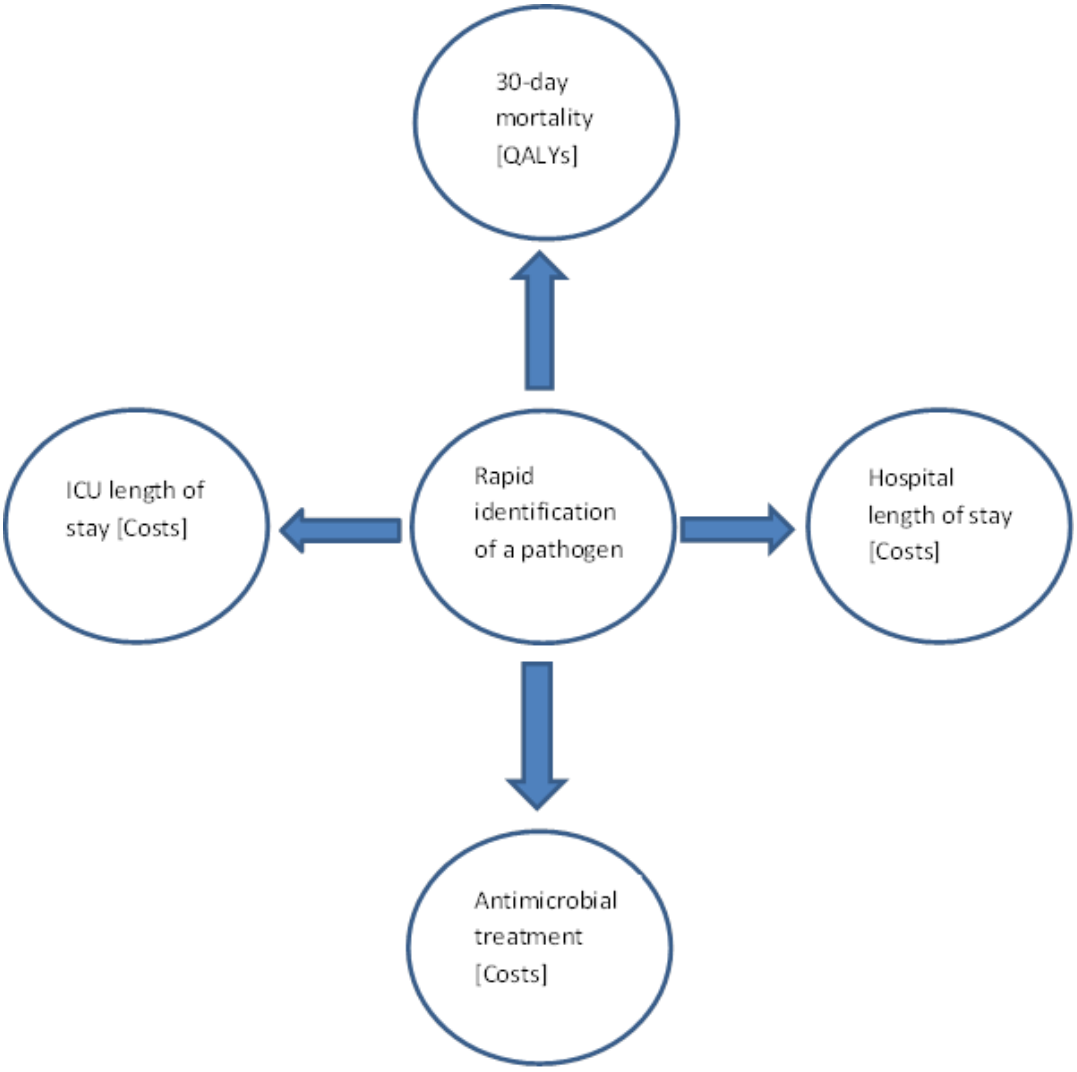
The External Assessment Group developed a de novo conceptual mathematical model designed to explore the cost effectiveness of the

LightCycler SeptiFast Test MGRADE, SepsiTest and the IRIDICA BAC BSI assay. The population included in the model is hospitalised patients with suspected bloodstream infection.

Model structure

The model comprised a decision tree with a lifetime time horizon and took the perspective of the NHS and Personal Social Services. The model is described in further detail on pages 97-100 of the diagnostics assessment report. The key clinical outcomes included the model are shown below in figure 4.

Figure 4 components within the de novo conceptual model



Model inputs

Data on the diagnostic accuracy of the interventions, intermediate outcomes and clinical outcomes were taken from the clinical effectiveness systematic review where possible. In addition expert opinion was sought to populate key clinical outcomes and supplement the data available from the systematic review. Routine sources of costs and prevalence data were also used where appropriate. A discount rate of 3.5% per annum was applied to both costs and effects. The potential impact of the tests on antimicrobial stewardship was not included in the model because of insufficient evidence to indicate how the

tests would impact upon antimicrobial use. Further details of the model inputs can be found starting on page 100 of the diagnostics assessment report.

Costs

The incremental cost per test was calculated using the cost of the test, the net effect on ICU and hospital length of stay, and changes in the costs of antimicrobial treatment. The cost per day for an ICU bed was estimated to be £1057 and for a general ward bed £275. A course of empirical antimicrobial treatment was estimated to cost £350.

It is assumed that cost per test is dependent upon both test throughput and whether laboratory equipment needs to be purchased to use the tests. The range of technology costs included in the model are shown below in table 2. No costs for blood culture were included in the model because this is common to all technologies. Full details of the costs of the interventions and comparators can be found starting on page 101 of the diagnostics assessment report.

Table 2 technology costs

Technology	Range of average costs
LightCycler SeptiFast Test MGRADE	£153.67 to £205.54
SepsiTest	£108.30 to £149.53
IRIDICA BAC BSI	£197.35 to £314.61
MALDI-TOF MS	£6.94 to £232.39.

Health related quality of life and QALY decrements

Incremental QALYs were calculated by assuming 11.32 discounted QALYs per 30 day mortality avoided, based on the estimated number of discounted life years for an adult patient with sepsis and the estimated quality of life after an episode of sepsis. The model assumed a mean age of 58 years and that 60% of the cohort were male. Patients were assumed to have a utility value of 0.68 at 5 years after an episode of severe sepsis (Cuthbertson et al. 2013)

unless the utility value predicted for the general population for the age and sex profile of the patient was lower.

Economic analysis results

For the purposes of decision making, the ICERs per QALY gained or lost will be considered. Five deterministic analyses were done:

1. Base case 1: interventions compared with blood culture with clinical outcome data taken from the systematic review
2. Base case 2: interventions compared with blood culture with clinical outcome estimates taken from expert opinion
3. Threshold analyses
4. Interventions compared with MALDI-TOF MS
5. Data taken from studies comparing more than one intervention

The following assumptions were common to all analyses:

- The only parameter to affect QALY gain or loss is 30 day mortality rate.
- Negative rapid tests do not impact upon any of the 4 key outcomes.
- Failed rapid tests do not impact upon any of the 4 key outcomes.
- Where 2.4 tests per day are run, laboratories run tests Monday to Friday only, with 3 times the number of tests run on Monday to account for sample accrual over a weekend.
- Where 17 or 68 tests per day are run, laboratories perform 3 runs per day and operate 24 hours a day 7 days a week.
- The purchase cost of machines required for the interventions and comparators is equally divided over 7 years of use.
- It is assumed that no additional staff costs or laboratory estate costs are incurred when using the interventions.

- The time scale of testing is 1 year although discounted QALYs accrued in subsequent years are included.
- Incremental QALYs are accrued through the number of avoided 30 day mortalities
- Where accuracy data from Warhurst et al. (2014) are used, the LightCycler SeptiFast Test MGRADE has a failure rate of 6.9%. A failure rate of 1.4% is assumed when pooled accuracy data is used.
- IRIDICA BAC BSI has a failure rate of [redacted]
- SepsiTest has a failure rate of 0%
- Patients are treated with either 18g per day of piperacillin/tazobactam or 3g per day of meropenem for 7 days.
- 30 day mortality rate is 13% unless otherwise specified.
- MALDI-TOF MS is only used on positive samples (8.7% of all blood cultures).
- MALDI-TOF MS has a sensitivity of 79.8% at species level compared with blood culture.
- LightCycler SeptiFast test MGRADE diagnostic accuracy data is derived from Warhurst et al. (2014) unless otherwise specified.
- SepsiTest and IRIDICA BAC BSI diagnostic accuracy data is derived from the External Assessment Group's meta-analyses unless otherwise specified.

Base case 1 results

In this analysis clinical outcome data derived from the clinical effectiveness review were included. This results in no clinical benefits in terms of 30 day mortality, length of stay in the intensive care unit or length of stay in hospital. The costs of antimicrobials are also unchanged in this analysis. All interventions are compared with blood culture only.

The results shown in table 3 are based on 2.4 tests per day. As the cost per test drops under increasing throughput the interventions remain dominated because of the lack of QALY gain.

Table 3 base case 1 results interventions compared with blood culture

	Incremental cost per test	Incremental QALYs per test	ICER
SeptiFast + necessary equipment	£205.54	0	Dominated
SeptiFast only	£201.23	0	Dominated
SepsiTest + necessary equipment	£149.53	0	Dominated
SepsiTest only	£142.48	0	Dominated
IRIDICA BAC BSI + necessary equipment	£314.61	0	Dominated
IRIDICA BAC BSI only	£270.89	0	Dominated

In addition, a threshold analysis was done for base case 1 to assess the reduction in antimicrobial costs that would be required for each intervention to be cost neutral. The results are shown below in table 4.

Table 4 reductions in antimicrobial costs required for the interventions to be considered cost neutral

	Required reduction in antimicrobial costs		
	2.4 tests run per day	17 tests run per day	68 tests run per day
SeptiFast + necessary equipment	59%	46%	44%
SeptiFast only	57%	46%	44%
SepsiTest + necessary equipment	43%	32%	31%
SepsiTest only	41%	32%	31%
IRIDICA BAC BSI + necessary equipment	90%	58%	58%
IRIDICA BAC BSI only	77%	56%	56%

Base case 2 results

In this analysis the key clinical outcome parameters were populated using an average of estimated values provided by clinical experts. The range of parameter estimates used in this analysis can be found starting on page 115 of the diagnostics assessment report. The External Assessment Group used these values in a range of scenarios which assume a 30 day mortality rate of either 13% or 29%, a throughput of 2.4, 17 or 68 tests per day and a maximum acceptable ICER of £20,000 or £30,000 per QALY gained. The comparator used in this analysis was blood culture.

For each scenario the net monetary benefit of each intervention was estimated. Net monetary benefit is calculated assuming a fixed maximum acceptable ICER. A positive net monetary benefit suggests that the benefits associated with the intervention outweigh the costs, and the intervention with the largest net monetary benefit is estimated to be the most cost effective. MALDI-TOF MS was also included in the analysis to estimate the relative cost-effectiveness between the two comparators included in the assessment.

In all scenarios modelled MALDI-TOF MS produced a positive net benefit compared with blood culture. In one scenario (30 day mortality rate 13%, 2.4 tests per day, maximum acceptable ICER of £20,000 per QALY gained), SepsiTest has the highest net monetary benefit when it is assumed that the equipment necessary to run the test is purchased. The IRIDICA BAC BSI assay has the highest net monetary benefit when only the test reagents and consumables are purchased. In all other modelled scenarios the IRIDICA BAC BSI assay has the highest net monetary benefit. The results from base case 2 are presented starting on page 123 of the diagnostic assessment report.

ICERs were also calculated using the data derived from expert opinion and are shown below in table 5 for the scenario which assumes a 13% mortality rate.

Table 5 Base case 2 estimated cost per QALY with an assumed mortality rate of 13% compared with blood culture

	Incremental cost per year	Incremental QALYs	ICER
2.4 tests per day			
SeptiFast + necessary equipment	£67,878	6.88	£9862
SepsiTest + necessary equipment	-£15,963	9.72	Dominates
IRIDICA BAC BSI + necessary equipment	£73,501	13.96	£5264
17 tests per day			
SeptiFast + necessary equipment	£201,782	48.81	£4134
SepsiTest + necessary equipment	-£343,990	68.96	Dominates
IRIDICA BAC BSI + necessary equipment	-£168,633	99.01	Dominates
68 tests per day			
SeptiFast + necessary equipment	£652,257	195.22	£3341
SepsiTest + necessary equipment	-£1,470,568	275.82	Dominates
IRIDICA BAC BSI + necessary equipment	-£674,533	396.06	Dominates

When it is assumed that no additional equipment is required to be purchased or the 30 day mortality rate is 29% the ICERs become more favourable because of either a reduction in incremental costs or an increase in incremental QALY gain compared with the scenario presented above in table 5. Full results of these analyses can be found starting on page 129 of the diagnostics assessment report.

In addition the External Assessment Group explored the impact of applying the pooled estimates of sensitivity and specificity derived from the meta-analyses to the LightCycler SeptiFast Test MGRADE. This assumption produced more favourable ICERs for the Light Cycler SeptiFast Test MGRADE through increasing the estimated sensitivity of the test (65% pooled estimate versus 51% Warhurst et al. 2015), whilst maintaining specificity at 86%. Further details of this analysis can be found on page 131 of the diagnostics assessment report.

Threshold analyses

The External Assessment Group used a range of threshold analyses to explore the impact of key clinical outcomes. The thresholds reported were as follows:

- Net reduction in mortality and net reduction in ICU length of stay
- Net reduction in antimicrobial costs and net reduction in ICU length of stay (driven solely by cost)

In all analyses it is assumed that the comparator has been purchased but the interventions need to be bought. The full results from the threshold analyses can be found in appendix 8 of the diagnostics assessment report. The threshold levels resulting from the analyses which assume 2.4 tests run per day and a maximum acceptable ICER of £20,000 per QALY gained are shown below in table 6. The values reported assume no change in either of the two remaining parameters. The threshold analyses which assumed either 17 or 68 tests run per day produced lower threshold values than those shown in table 6. In addition, required values were lower when a maximum acceptable ICER of £30,000 per QALY gained is assumed.

Table 6 threshold levels required for a cost per QALY gained of £20,000 (assuming 2.4 tests per day)

	Per 100 tests			Per 100 positive tests		
	Reduction in 30 day mortality (lives)	**Reduction in ICU stay (days)**	**Reduction in antimicrobial costs (£)**	**Reduction in 30 day mortality (lives)**	**Reduction in ICU stay (days)**	**Reduction in antimicrobial costs (£)**
Compared with blood culture						
SeptiFast	0.09	19.45	205.54	0.62	133.82	1414.50
SepsiTest	0.07	14.15	149.53	0.39	83.46	882.15
IRIDICA BAC BSI	0.14	29.76	314.61	0.65	140.23	1482.28
Compared with MALDI-TOF						
SeptiFast	0.09	18.50	195.57	0.59	127.33	1345.90
SepsiTest	0.06	13.20	139.56	0.36	77.89	823.34
IRIDICA BAC BSI	0.13	28.82	304.65	0.63	135.79	1435.25

Cost effectiveness of the LightCycler SeptiFast Test MGRADE and SepsiTest compared with MALDI-TOF MS

The External Assessment Group also explored the cost effectiveness of both the LightCycler SeptiFast Test MGRADE and SepsiTest compared with MALDITOF-MS using data from two studies (Tafelski et al. 2015 and Loonen et al. 2014) which included MALDI-TOF-MS in addition to blood culture. The effect estimates based on expert opinion were also included in the analysis. The results of these analyses suggest that the LightCycler SeptiFast Test MGRADE dominates MALDI-TOF MS and SepsiTest has ICERs ranging from £23,375 to £34,848 per QALY gained with a 30 day mortality rate of 13% and from £10,479 to £15,621 per QALY gained with a 30 day mortality rate of 29%. These results are in contrast to those of base case 2, which suggest that SepsiTest is more cost-effective than both MALDI-TOF MS and the LightCycler SeptiFast Test MGRADE. Full results of this analysis can be found starting on page 139 of the diagnostics assessment report.

Results from studies comparing the LightCycler SeptiFast Test MGRADE and SepsiTest simultaneously with blood culture

An analysis was run using data from two studies (Schreiber et al. 2013 and Leitner et al. 2013) which evaluated both the LightCycler SeptiFast Test MGRADE and SepsiTest with blood culture. The analysis was done to compare the relative cost-effectiveness estimates with those derived in base case 2 which were based on indirect comparisons of the relative effectiveness of the interventions from expert opinion. The analysis assumes a 0% test failure rate for both interventions. Full results of the analysis can be found on pages 143-144 of the diagnostics assessment report. A range of scenarios were presented with 30 day mortality rates of 13% or 29% and a throughput of 2.8, 17 or 68 tests per day. In all scenarios the ICER for the LightCycler SeptiFast Test MGRADE was greater than £30,000 per QALY gained when compared with SepsiTest. The results are concordant with base case 2, where SepsiTest had a higher net monetary benefit than the LightCycler SeptiFast Test MGRADE.

3 Key findings from assessment

- The IRIDICA BAC BSI assay is estimated to have greater sensitivity (81%) than the LightCycler SeptiFast Test MGRADE (65%) or SepsiTest (48%), although each of the interventions are estimated to have similar specificity (84%- 86%) when compared with blood culture. The studies from which the diagnostic accuracy data were drawn are subject to limitations which impact upon the reliability of the pooled accuracy data. No evidence was found to suggest that the use of the interventions will have an impact on patient outcomes such as intensive care unit and hospital length of stay and changes in antimicrobial treatment plans, although expert opinion suggests that clinicians believe the interventions could produce clinical benefits.
- There is considerable uncertainty in the economic analyses because of insufficient clinical outcome data. It is plausible that the assumed higher sensitivity of the IRIDICA BAC BSI assay could be associated with an increase in QALYs and in cost reductions from intensive care unit and hospital lengths of stay and in changes in antimicrobial costs, although this is the intervention associated with the greatest cost. SepsiTest typically had a higher net monetary benefit than the LightCycler SeptiFast Test MGRADE despite having lower sensitivity, but this comparison was driven by an assumed lower cost per test and also a greater estimated benefit of the test provided by expert opinion. Threshold analyses suggested that only small mortality gains are needed for the ICER to be less than £20,000 per QALY gained, but it is highly uncertain whether this would be achieved in practice.

4 Issues for consideration

Clinical effectiveness

- The studies included in the clinical effectiveness review are considered to be at risk of bias with the studies including populations with differing

demographics, different blood sample volumes, differences in administration of empiric antimicrobials, and different definitions of true positive results. The External Assessment Group noted that the majority of the studies had deficiencies in reporting and study quality, which impact upon the reliability of the reported effect estimates.

- The included studies were also noted to have concerns regarding applicability particularly with regards to whether the results address the decision problem and can be generalised to current UK practice. In particular all of the included studies did not provide adequate details of the reference standard test and it is uncertain whether there are differences in blood culture sampling, processing and analysis. Further, only 31 studies reported running the interventions in accordance with the CE marked test protocol.
- Two of the 4 studies which report diagnostic accuracy data for the IRIDICA BAC BSI assay included an older version of the PCR/ESI-MS analyser known as the PLEX-ID. This included desalter and mass spectrometry modules which differ to those in the currently available IRIDICA PCR/ESI-MS analyser.
- All of the included SepsiTest studies report data for versions of the test which are no longer available. A fourth version of the SepsiTest has been released by the company and it is not certain whether the results presented in the diagnostics assessment report are applicable to the current version.
- There is considerable heterogeneity in the included diagnostic accuracy studies which is highlighted by the large credible and prediction intervals around the pooled accuracy estimates. This may be driven by the differences in patient populations included in the studies, although meta-regression for the Light Cycler SeptiFast Test MGRADE analysis suggested that the pooled effect estimates were not influenced by the age of patients, exposure to antibiotics prior to blood sample collection, community or hospital acquired infection, inclusion of patients with febrile neutropenia or whether contaminants were included in the reported results.

- All estimates of diagnostic accuracy for the interventions assume that the reference standard, blood culture, is 100% sensitive and specific. However in practice a number of factors are known to influence the accuracy of blood culture and the estimates of sensitivity and specificity may therefore be underestimated. In addition, the parameter estimates derived from clinical opinion in the economic evaluation suggested that when the intervention was positive and the blood culture negative the experts believed that the discordance was likely to be in favour of the interventions, that is the molecular test is a true positive and the blood culture a false negative.
- Studies reported data using different units of analysis, for example accuracy reported for patients, sample episodes or species/pathogen level. This has created a unit of analysis error in the meta-analyses which leads to inaccuracy in the pooled estimates.
- Only limited data were available comparing the LightCycler SeptiFast Test MGRADE and SepsiTest with MALDI-TOF MS and blood culture. No data were available to allow a comparison with the IRIDICA BAC BSI assay. The relative accuracy and effectiveness of the interventions with MALDI-TOF MS and blood culture is therefore highly uncertain.
- In clinical practice, it is possible that patients may present with polymicrobial infection. The impact of this may not have been fully captured in the analysis.
- Only limited data were found on the impact or safety of acting on the results of the interventions in practice. Where data were reported for intermediate and/or clinical outcomes they were generally reported on a cohort level and comparisons between the index test and reference test could not be done. Further, it was often unclear how long patients were followed up for clinical outcome events.
- None of included studies provided data on re-admission rates, adverse events associated with broad spectrum antimicrobial use, morbidity,

changes in disease severity over time, rates of superinfection, rates of resistant infection or health related quality of life.

Cost effectiveness

- The results of the health economic analyses are highly uncertain. This is primarily because of insufficient data on the impact of the interventions on patient outcomes such as sepsis-related mortality, length of stay in the intensive care unit and changes in the costs of antimicrobial therapy. Where impacts on patient outcomes are included the model, the estimates are driven by values obtained from clinical experts. In addition, the pooled diagnostic accuracy results used in the analyses assume that blood culture is 100% sensitive and specific.
- There are considerable differences in the results produced by the two base cases. In base case 1 all interventions are dominated because there were no data to suggest that any knowledge gained from the rapid tests translate into patient benefits; however, in base case 2 clinical opinion suggests that the use of the rapid tests could translate into clinical benefit. Despite the general belief that the tests could translate into clinical benefit there was wide variation in estimated values between the 7 clinical experts. This discordance suggests that the calculated ICERs for the interventions are highly uncertain.
- The QALY gains included in the model are calculated based on an adult with sepsis. It is plausible that the QALY gains for children and neonates would be greater due to longer life expectancy, particularly for neonates, although the magnitude of the difference is uncertain and there is insufficient clinical data to model these populations.
- There is uncertainty around the test failure rate estimates included in the model. There is wide variation in the estimates derived from both the LightCycler SeptiFast Test MGRADE and IRIDICA studies, and no data are available for SepsiTest. It is possible that the failure rates could have a substantial impact on the relative incremental costs between the interventions if extreme values are used.

- The relative incremental cost effectiveness data available for comparing the interventions to one another is highly uncertain. The IRIDICA BAC BSI is assumed to have greater sensitivity than both the LightCycler SeptiFast test MGRADE and SepsiTest which results in increased QALY gain and health system savings which are sufficient to offset the more expensive costs of the intervention and provide the highest net monetary benefit in base case 2. SepsiTest also has higher net monetary benefit than the LightCycler SeptiFast Test MGRADE despite being less sensitive, but this comparison is driven by a lower cost and a greater benefit estimated from expert opinion.
- The cost-effectiveness of the interventions compared with MALDI-TOF MS could not be fully assessed with the data available. Two studies compared the LightCycler SeptiFast Test MGRADE or SepsiTest with MALDI-TOF MS and were used in analysis 4 which suggested that the LightCycler SeptiFast Test MGRADE is more cost effective than MALDI-TOF MS. However this result contradicts the findings of base case 2 where SepsiTest appeared to be more cost effective than both the LightCycler SeptiFast Test MGRADE and MALDI-TOF MS.
- The External Assessment Group highlighted that the following variables have not been captured in the economic analyses, although it is not believed that these would impact on the conclusions in light of the uncertainties in clinical outcomes:
  - antimicrobial stewardship benefits
  - cost implications of any service reconfiguration required to move to testing 24 hours a day 7 days a week
  - training costs required
  - any utility differential between survivors with and survivors without any intervention
  - the possibility that only a sequencer needs to be purchased to run SepsiTest
  - discounts associated with processing large volumes of tests

5 Equality considerations

NICE is committed to promoting equality of opportunity, eliminating unlawful discrimination and fostering good relations between people with particular protected characteristics and others.

Bloodstream infection may be a particular risk for neonates, older people, people who are immunocompromised and pregnant women. People with cancer are at risk of neutropenic sepsis.

Tests that need higher volumes of blood for a sample may be less suitable for use in neonatal and paediatric patients.

6 Implementation

The adoption of direct sample whole blood molecular tests may require changes to laboratory processes and workflow to achieve rapid turn-around times for processing and reporting samples. It may also be difficult to obtain the volume of blood required for a sample for direct whole blood molecular testing in some critically ill neonates and paediatric patients.

7 Authors

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August 2015

Appendix A: Sources of evidence considered in the preparation of the overview

A. The diagnostics assessment report for this assessment was prepared by School of Health and Related Research (ScHARR), University of Sheffield.

Stevenson M, Pandor A, Martyn-St James M et al. Sepsis: The LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi. A systematic review and economic evaluation. July 2015.

B. The following organisations accepted the invitation to participate in this assessment as stakeholders. They were invited to attend the scoping workshop and to comment on the diagnostics assessment report.

Manufacturer(s) of technologies included in the final scope:

- Abbott Laboratories
- Roche Diagnostics
- Molzym

Other commercial organisations:

- Alacrita LLP
- Anagnostics
- Hain Lifescience UK Ltd

Professional groups and patient/carer groups:

- Group B Strep Support
- UK Sepsis Trust

Research groups:

none

Associated guideline groups:

- National Clinical Guidelines Centre

Others:

- Department of Health
- Healthcare Improvement Scotland
- NHS England
- Welsh Government

Appendix B: Glossary of terms

Anaerobic bacteriaemia
Bloodstream infections caused by anaerobic bacteria. Anaerobic bacteria are the most common flora in the body but may cause serious infection after injury or trauma to the body. Anaerobic bacteria include Gram Positive and Gram Negative cocci and rods.

Bivariate
Data that has two variables. In this instance the variables (sensitivity and specificity) are related.

Broad spectrum antibiotic
An antibiotic which is effective against a broad range of bacteria. Broad spectrum antibiotics typically include coverage against gram-positive and gram-negative bacteria.

Carbapenems
Broad spectrum antibiotics which are often used as the last line of treatment for hard to treat human infections caused by gram-negative bacteria.

Carbapenemases
Enzymes produced by bacteria that destroy carbapenems and other beta-lactam antibiotics.

Disc diffusion method
A method of antimicrobial susceptibility testing which involves placing antimicrobial impregnanted discs onto an agar plate containing bacterial cultures. If the antibiotic is effective against the bacteria, there will be a visible zone which is devoid of bacterial growth surrounding the disc.

Empiric antibiotic
An antibiotic given to a person before a specific microorganism or source of the potential infection is known. It is usually a broad-spectrum antibiotic and the treatment may change if the pathogen or source is confirmed.

Extended-spectrum beta-lactamases
Enzymes produced by bacteria making them resistant to penicillins and cephalosporins.

Gram-negative bacteria
Bacteria that do not retain crystal violet dye in the Gram-staining procedure.

They can cause many types of infection and include *E. coli* and *Pseudomonas aeruginosa.*

Gram-positive bacteria
Bacteria that are stained dark blue or violet in the Gram-staining procedure. They include *Stapyhlococcus aureus* and *Clostridium difficile.*

Healthcare associated infections
Infections acquired via the provision of healthcare in either a hospital or community setting.

MALDI-TOF mass spectrometry
MALDI-TOF (matrix-absorbed laser desorption/ionization- time of flight) mass spectrometry may be used to identify bacteria and fungi from positive blood cultures.

Markov Chain Monte Carlo simulation
A method for estimating the values of parameters in a mathematical model.

Meticillin-resistant *Staphylococcus aureus* (MRSA)
A strain of Staphylococcus aureus that is resistant to beta lactam antibiotics which include penicillins (e.g. meticillin and oxacillin) and almost all cephalosporin antibiotics.

Neutropenia
An abnormally low number of neutrophils which are a type of white blood cells that help to fight off infections by destroying bacteria and fungi. People who have neutropenia are therefore at an increased risk of developing a serious infection.

Polymicrobial infection
An infection which is caused by more than one pathogen and which may include a combination of bacteria, fungi and viruses.

Sepsis
A life-threatening systemic inflammatory response caused by the presence of an infectious agent (i.e. bacterial, viral, fungal or parasitic).

Severe sepsis
A septic infection that is associated with signs of organ dysfunction, damage and altered cerebral function leading to septic shock. Most patients with severe sepsis require treatment in intensive care units and severe sepsis can lead to death.

Septic shock
Sepsis-induced hypotension persisting despite adequate fluid resuscitation.

Superinfection
A new infection occurring in a patients with a pre-existing infection.

Systemic Inflammatory Response Syndrome
A life-threatening condition which arises from a severe systemic response to either an infectious or non-infectious insult.

NICE National Institute for Health and Care Excellence

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Diagnostics Assessment Report (DAR) - Comments

Stakeholder	Comment no.	Page no.	Section no.	Comment	External Assessment Group response
Abbott	1.	93	3.1.2.3	Clarification on Bilkovski et. al. model: the assumptions for this model were analogous to the HEOR model assumptions presented by NICE (p99), i,e., only positive detections by IRIDICA were taken into account for assuming changes in patient management. No predictions were made based on Negative IRIDICA results. While it is true that this IRIDICA testing is different from the MALDI-TOF analysis that is based on positive cultures only, for our model, we assumed that IRIDICA results were true even in the absence of corresponding culture positivity. This assumption is consistent with the NICE model assumptions stated throughout the document which indicate culture could be negative 30-60% of the time (p13, sec 1.4.1), and how clinical experts involved in this review supported the fact that they would trust the intervention result over a culture negative result (p113, sec 3.4.3)	No response needed
	2.	109	3.4.1.2.4	The cost analysis of IRIDICA is made just on BAC BSI, but the same platform can be used for detecting other pathogens such as fungi and viruses and can be used with other sample matrices: ETA, BAL, SFT, etc. This should perhaps be taken into consideration, similar to the comment on MALDI-TOF on p109 where only 50% of the costs are attributed to sepsis management	We agree that if IRIDICA was used for detection in areas other than sepsis then the cost per test within sepsis would decrease. However the advice provided by our clinical advisors was that 100% of the costs apportioned to sepsis was appropriate.

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Stakeholder	Comment no.	Page no.	Section no.	Comment	External Assessment Group response
	3.	112	3.4.1.7	RADICAL study across 6 European countries and 9 sites had a mean usage of 3.2 antibiotics per patient, with the 2 UK sites averaging 1.99 Ab per patient. We feel the contribution of the assumed empirical therapy may be underestimated in the model presented here	This is noted, although our base case was believed to be reasonable by our clinical experts. Whilst increased treatment costs are likely to be favourable to the interventions the gain is small compared with changes in the ICER due to the uncertainty in clinical effectiveness.
	4.	112	3.4.1.8	RADICAL study had an observed 30-day mortality of ~30%, similar to Mouncey et. al. The patient population was critically ill and may account for the difference compared to HTA reported rates, but we felt it was important to point out.	Unclear of the point being made. We have undertaken scenario analyses using a 29% mortality rate should the Committee wish to use this value.
	5.	145	3.4.5	Would NICE recommend another UK body lead and fund the study they suggest is required?	Not a question for the External Assessment Group (although we were not sure of the exact part of the report being referenced)
	6.	152	5.3.2	Confirm reference to Section 5.4 in Conclusion is a typo and not a missing section on recommended studies- we didn't receive a section 5.4	Yes, this is a typo (and is also made in the last sentence on page 152). This should refer to Section 5.2
Group B Strep Support	1.	1	General	We welcome this report and are pleased that consideration is being given to methods for the rapid identification of bloodstream bacteria and fungi. Intrapartum GBS sepsis is a risk for the mother as well as the baby, and so a rapid test that could detect the onset of septicaemia would be of considerable value.	No response required

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Stakeholder	Comment no.	Page no.	Section no.	Comment	External Assessment Group response
	2.	vi	Plain English Summary	The conclusions seem logical given the evidence reviewed. We note the conclusion that in order to provide better estimates, studies should be undertaken where information from the tests is allowed to change clinical practice and for these results to be compared with those from current practice. We hope and trust that funders will be actively encouraged to support such research studies, so that speedy progress can be made to improve the targeting of appropriate antibiotics.	No response required
Molzym	1.	**104**	**3.4.1.2.2**	***SepsiTest*™ is based on two basic steps:** **Step 1:** Pathogen DNA isolation and purification **Step 2:** a. Analysis of the eluate by universal rDNA PCR for pathogen DNA b. Sequence analysis of amplicons and identification of pathogens **Controls for Step 1:** <u>Negative extraction control:</u> We recommend running one control with each batch	It is unclear whether this is a comment intended at receiving an External Assessment Group response.

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				of samples in order to test for potential cross-contamination during sample extraction. For the negative extraction control, buffer SU can be used which is provided with the kit. All PCR reagents needed to analyse the negative extraction control are provided with the kit. Positive extraction control: For new customers we suggest to calibrate the procedure with spiking negative samples (e.g. whole blood or buffer SU) with dilutions of full-grown cultures of pathogens or by using e.g. BioBall MultiShot 550 KBE (bioMerieux). The positive sample control is not provided with the kit. All PCR reagents needed to analyse the positive extraction control are provided with the kit. **Controls for Step 2:** Negative PCR control DNA-free water is supplied with the kit as PCR negative control and is added to each assay instead of eluate (target DNA) for each sample batch. The control is meant to detect any exogenous DNA coming in as carry-over or handling contamination during PCR setup.	

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				All PCR reagents are provided with the kit. Positive PCR control A concentrated DNA standard (P1) is supplied with the kit as PCR positive control to make sure that the PCR assays are performing as specified. The set of controls comprises of a high (P1) and low (P2) standard DNA for Mastermix Assay Bacteria (MA Bac) and Mastermix Assay Yeasts (MA Yeasts). The high standard DNA (P1) indicates the functioning of the assays. The low concentrated DNA standard (P2) is a test for the sensitivity of the assays. Positive PCR controls P1 and P2 have to be performed with each set of analyses. All PCR reagents are provided with the kit.	
	2.	**105**	**3.4.1.2.2**	**Costs for required equipment for SepsiTest™ are listed in table 21 (not in table 19)**	Agreed. This should refer to Table 21
Royal College of Pathologists	1.	**114-118**	**3.4.3**	Wide variations from the clinicians estimates were used to calculate the net monetary benefit from the 3 interventions. Notably it is difficult to understand the	No response needed

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Stakeholder	Comment no.	Page no.	Section no.	Comment	External Assessment Group response
				wide variation in cost benefit between interventions. For example clinician 7 estimates wide variation in savings in antimicrobial costs between interventions with no justification for these differences. Based on published studies (Base case 1) there was no cost benefit observed by employing any of the 3 interventions, where documented statistically significant benefits of the tests were used alone. There was a cost benefit when the views of the clinicians regarding perceived benefits were taken into account (Base case 2), however as the authors point out these views varied widely between clinicians and consequently these calculations should be used with caution. There is clearly a need for more robust studies to inform on the clinical and cost effectiveness of these interventions	
	2.	**114-118**	**3.4.3**	Wide variations from the clinicians estimates were used to calculate the net monetary benefit from the 3 interventions. Notably it is difficult to understand the wide variation in cost benefit between interventions. For example clinician 7 estimates wide variation in savings in antimicrobial costs between interventions with no justification for these differences. Based on published studies (Base case 1) there was no cost	No response needed

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Diagnostics Assessment Report (DAR) - Comments

Stakeholder	Comment no.	Page no.	Section no.	Comment	External Assessment Group response
				benefit observed by employing any of the 3 interventions, where documented statistically significant benefits of the tests were used alone. There was a cost benefit when the views of the clinicians regarding perceived benefits were taken into account (Base case 2), however as the authors point out these views varied widely between clinicians and consequently these calculations should be used with caution. There is clearly a need for more robust studies to inform on the clinical and cost effectiveness of these interventions	
	3.	**145**		The External Assessment Group comment that studies comparing the use of an intervention with standard practice, where the results from the tests are fed into a treatment management plan, are urgently needed to produce more definitive estimates of the cost per QALY gained. The RAPIDO study is undertaking this for MALDI-TOF MS in addition to blood culture and clinical judgement.	We agree, and have mentioned this on pages 146 and 152, where we state that 'Any such trials should wait until the results from the RAPIDO trial are published in order that key information on the clinical utility of MALDI-TOF MS compared with blood culture is known'.
	4.	**106**		Table 21 describes the costs of running the SepsiTest and details the use of a refurbished ABI 310 instrument at cost of 30 000 Euros. It is likely	Comment noted. Whilst the capital costs of the machinery required does influence the ICER these changes are relatively small

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				this is an underestimate of sequencing costs as this instrument will not be supported by Life technologies in the future. It is single capillary instrument which limits throughput. It is perhaps more likely that a laboratory that has sequencing capability and expertise would use existing equipment that was already in place for other sequencing commitments. This would however create pressure on access to the instrument where sequencing of SeptiTest requires prompt access to identify positive samples.	compared with changes in the ICER due to the uncertainty in clinical effectiveness.
	5.	**102**		The authors assume that there would be no additional room or staff costs incurred by purchasing one of these interventions. All of the 3 methods are quite labour intensive and require a reasonably high level of technical expertise particularly for implementing sequencing methodology. These interventions are likely to offer the maximum benefit when results are available as early as possible. This therefore limits the potential for batching samples and clearly there would be benefits from running these assays on a 24 hour 7 day basis. It is also likely there would be pressure on laboratory space where distinct laboratory space is required for sample extraction, amplification and detection and nucleotide sequencing.	Comment noted. Whilst the costs associated with staffing and accommodation would influence the ICER these changes would be relatively small compared with changes in the ICER due to the uncertainty in clinical effectiveness.

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Diagnostics Assessment Report (DAR) - Comments

Stakeholder	Comment no.	Page no.	Section no.	Comment	External Assessment Group response
				Centralisation of testing to a regional hub laboratory may offer a cost effective and efficient use of staff expertise, however there would inevitably be delays in transportation that may reduce the benefits of obtaining a rapid result.	
	6.			The meta analyses showed specificity to be superior to sensitivity. The authors comment on the potential for bias and the wide heterogeneity of the published data. There may be problems using blood culture as the gold standard as there are potentially a number of confounding factors such as pre-sampling use of antibiotics and sample timing, however the figures for sensitivity appear quite disappointing (SeptiFAST 68%; SeptiTest 48%, and IRIDICA 81%. This does indicate there were a substantial number of blood culture positive isolates not detected by these 3 interventions. There are a limited number of organisms that are detected by these PCR methods (particularly with SeptiFast) and blood culture may therefore identify other more unusual organisms not included in the repertoire of organisms identified. This possibility was not investigated in this report. Specificity was higher for all 3 tests although it is unlikely that a negative result alone would result in a change of therapy although would be useful to	This does not appear a comment to which the External Assessment Group need respond.

Sepsis: The LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi

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				support a subsequent blood culture negative result.	
Roche	1.	**121**	**3.5.2**	The presented mathematical model seems a reasonable framework to estimate the value of SeptiFast and alternative technologies in providing diagnostic information earlier than current methods. The analysis results based on clinical opinion (base case 2), indicate that use of SeptiFast could be cost effective. This seems to be supported by the presented threshold analysis. However, due to the absence of robust head-to-head data it is in our opinion not feasible to draw conclusions on the relative cost-effectiveness of difference index tests (interventions).	No comment required
	2.	**144**	**3.6**	We strongly agree with the caution against comparing results on diagnostic accuracy, clinical effectiveness or cost effectiveness of the index tests (interventions). Such comparisons are indirect and are likely to be biased.	No comment required
	3.	**146**	**4.1.1**	We like to highlight that sensitivity & specificity values for SeptiFast in this report were derived from a meta-analysis including a significant number of studies and patients.	No comment required
	4.	**148**	**4.2.1**	We agree with the observation that blood culture is likely to be an imperfect reference standard as highlighted in Warhurst et al. 2015, leading to a systematic under-estimation of sensitivity and	No comment required

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				specificity. This issue may also explain the heterogeneity of diagnostic accuracy studies in the meta-analysis.	