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

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NHS

National Institute for Health Research

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This report

Abstract

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

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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® (Roche Diagnostics, Risch-Rotkreuz, Switzerland); SepsiTest™ (Molzym Molecular Diagnostics, Bremen, Germany); and the IRIDICA BAC BSI assay (Abbott Diagnostics, Lake Forest, IL, USA)] 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/ionisation time-of-flight mass spectrometry).

Data sources: Thirteen electronic databases (including MEDLINE, EMBASE and The Cochrane Library) were searched from January 2006 to May 2015 and supplemented by hand-searching relevant articles.

Review methods: A systematic review and meta-analysis of effectiveness studies were 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; all other parameters were estimated from published sources. The model was populated with evidence from the systematic review or individual studies, if this was considered more appropriate (base case 1). In a secondary analysis, estimates (based on experience and opinion) from seven clinicians regarding the benefits of earlier test results were sought (base case 2). A 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.

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 summary 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). Four studies comparing SepsiTest with blood culture found that SepsiTest had an estimated summary specificity of 0.86 (95% CrI 0.78 to 0.92) and sensitivity of 0.48 (95% CrI 0.21 to 0.74), and four studies comparing IRIDICA with blood culture found that IRIDICA had an estimated summary specificity of 0.84 (95% CrI 0.71 to 0.92) and sensitivity of 0.81 (95% CrI 0.69 to 0.90). Owing to the deficiencies in study quality for all interventions, diagnostic accuracy data should be treated with caution. No randomised clinical trial evidence was identified that indicated that any of the tests significantly improved key patient outcomes, such as mortality or duration in an intensive care unit or hospital. 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; the IRIDICA BAC BSI assay had the highest estimated incremental net benefit, but results from base case 2 should be treated with caution as these are not evidence based.

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

Conclusions: The clinical effectiveness 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: This study is registered as PROSPERO CRD42015016724.

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Glossary

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 Drugs used to treat infections, such as antibiotics and antifungal and antiviral drugs.

Bacteraemia 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.

Colony-forming unit A unit used to estimate the number of viable bacteria or fungal cells in a sample.

Conformité Européenne mark A manufacturer's declaration that the product meets the requirements of the applicable Electronic Commerce directives.

Dominated Of an intervention, more expensive and providing the same or fewer additional quality-adjusted life-years than another intervention, or equally expensive and providing fewer quality-adjusted life-years than another intervention.

Dominating Of an intervention, less expensive and providing the same or more quality-adjusted life-years than another intervention, or equally expensive and providing more quality-adjusted life-years than another intervention.

Empiric treatment A therapy based on clinical experience in the absence of complete information.

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.

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.

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 of which the 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 a one-unit increase in effectiveness.

Narrow-spectrum antibiotic An antibiotic effective against specific families of bacteria.

Nosocomial infection Hospital-acquired infection.

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.

Quality-adjusted life-year 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 the quality of life the person is predicted to have.

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.

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.

Specificity The proportion of people without the target condition who receive a negative 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.

Superinfection A second infection superimposed on an earlier one, especially by a different microbial agent of exogenous or endogenous origin, which is resistant to the treatment used against the first infection.

List of abbreviations

APACHE	Acute Physiology And Chronic Health Evaluation	NMB	net monetary benefit
CE	Conformité Européenne	PCR	polymerase chain reaction
cfu	colony-forming unit	QALY	quality-adjusted life-year
CI	confidence interval	QUADAS	Quality Assessment of Diagnostic Accuracy Studies
CrI	credible interval	RCT	randomised controlled trial
DNA	deoxyribonucleic acid	SAPS	Simplified Acute Physiology Score
EDTA	ethylenediaminetetraacetic acid	SD	standard deviation
ICER	incremental cost-effectiveness ratio	SIRS	systemic inflammatory response syndrome
ICU	intensive care unit	SIRS-SS	systemic inflammatory response syndrome with suspected sepsis
MAICER	maximum acceptable incremental cost-effectiveness ratio	SOFA	Sequential Organ Failure Assessment
MALDI-TOF	matrix-absorbed laser desorption/ionisation time of flight	VAT	value-added tax
MS	mass spectrometry		
NICE	National Institute for Health and Care Excellence		

Note

This monograph is based on the Technology Assessment Report produced for NICE. The full report contained a considerable number of data that were deemed confidential. The full report was used by the Appraisal Committee at NICE in their deliberations. The full report with each piece of confidential data removed and replaced by the statement 'confidential (or data) removed' is available on the NICE website: www.nice.org.uk.

The present monograph presents as full a version of the report as is possible while retaining readability, but some sections, sentences, tables and figures have been removed. Readers should bear in mind that the discussion, conclusions and implications for practice and research are based on all the data considered in the original full NICE report.

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.

This report looked at the clinical effectiveness and cost-effectiveness of three tests: LightCycler SeptiFast Test MGRADE® (Roche Diagnostics, Risch-Rotkreuz Switzerland); SepsiTest™ (Molzym Molecular Diagnostics, Bremen, Germany); and the IRIDICA BAC BSI assay (Abbott Diagnostics, Lake Forest, IL, USA). These tests are designed to provide the clinician with a result regarding whether or not a patient has sepsis and which bacterium or fungus is the cause, and are much quicker than current methods. A review of the published literature showed that the tests are better at correctly identifying patients without sepsis than those with sepsis, but that all three tests are imperfect.

However, because of limitations in reporting and study quality, these data should be treated with caution. A review of the published literature showed that the tests can decrease the time at which the clinician received the result and decrease the time at which some patients changed to a better treatment within clinical trials. However, these benefits may not be realised in clinical practice. Furthermore, key benefits (reduced mortality, reduced length of stay in intensive care units and hospital, and reduced costs of treatment) of the new tests had yet to be proven within clinical trials. 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.

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 patients with a suspected bloodstream infection or sepsis consists of clinical assessment in conjunction with blood culture [with or without matrix-absorbed laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS)]. However, positive blood culture results for bacteria or fungi can take several days. Several new tests have been developed that can detect minute amounts of pathogens' deoxyribonucleic acid in patients' whole-blood samples, with results available within approximately 6 hours under optimal conditions, although this time is likely to be increased if the tests are introduced routinely into clinical practice. It is noted that recently published guidelines, if followed, are likely to reduce the consequences of sepsis.

Objectives

To evaluate the clinical effectiveness and cost-effectiveness of three tests [LightCycler SeptiFast Test MGRADE® (Roche Diagnostics, Risch-Rotkreuz, Switzerland), SepsiTest™ (Molzym Molecular Diagnostics, Bremen, Germany) and the IRIDICA BAC BSI assay (Abbott Diagnostics, Lake Forest, IL, USA)] for the rapid identification of bloodstream 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) from January 2006 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. Data were extracted at the pathogen level, where reported in the published literature, otherwise data were extracted at the patient level. Results were summarised in tables and text. Data analysis comprised a narrative synthesis and pairwise 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, in which only published statistically significant data were used within the model; and base case 2, in which 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 (2.4–68) of blood samples that need analysing per day 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 antimicrobial drugs.

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 metaregression 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% confidence interval (CI) 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 a 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 because of 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 a sensitivity of 0.81 (95% CrI 0.69 to 0.90). However, there was substantial heterogeneity between studies. Moreover, owing 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, $n = 7$ (confidential information has been removed)]; 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, $n = 14$; and IRIDICA, $n = 1$); and mortality (SeptiFast, $n = 17$; SepsiTest, $n = 1$; IRIDICA, $n = 1$; and SeptiFast/SepsiTest, $n = 1$). 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 remain unclear. Of the comparative studies, a small number of low-methodological-quality randomised controlled trials (all SeptiFast studies) indicated no statistically significant between-group differences for SeptiFast compared with blood culture in length of hospital stay ($n = 3$), length of intensive care unit stay ($n = 2$), duration of antimicrobial therapy ($n = 1$) or mortality ($n = 3$).

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 was 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 < £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 multitest 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 the three tests with 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 on in clinical practice were an intervention introduced. This is because the sensitivity of the tests is not high enough to allow them to be used in a 'rule-out' manner, such that clinicians can be reassured that a negative test is associated with a very low probability of the patient having the disease. Furthermore, because of the deficiencies in study design and poor reporting of the included studies, test characteristic 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 with 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 metaregression. 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 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. Definitive data on this need 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 effectiveness or the 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.

Study registration

This study is registered as PROSPERO CRD42015016724.

Funding

Chapter 1 Background and definition of the decision problem

Background to sepsis and bloodstream 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 bloodstream 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 bloodstream, a process that 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*.

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 the presence of at least two SIRS criteria;[2] however, a recent paper suggests that the need for two or more SIRS criteria excludes one in eight patients with infection, organ failure and substantially increases mortality risk.[3] SIRS criteria are a fever of > 38 °C or < 36 °C; heart rate of > 90 beats per minute; respiratory rate of > 20 breaths per minute or arterial carbon dioxide tension of < 32 mmHg; or an abnormal white blood cell count [> 12,000 cells per μl or < 4000 cells per μl, or > 10% immature neutrophils (bands)].[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 and 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]
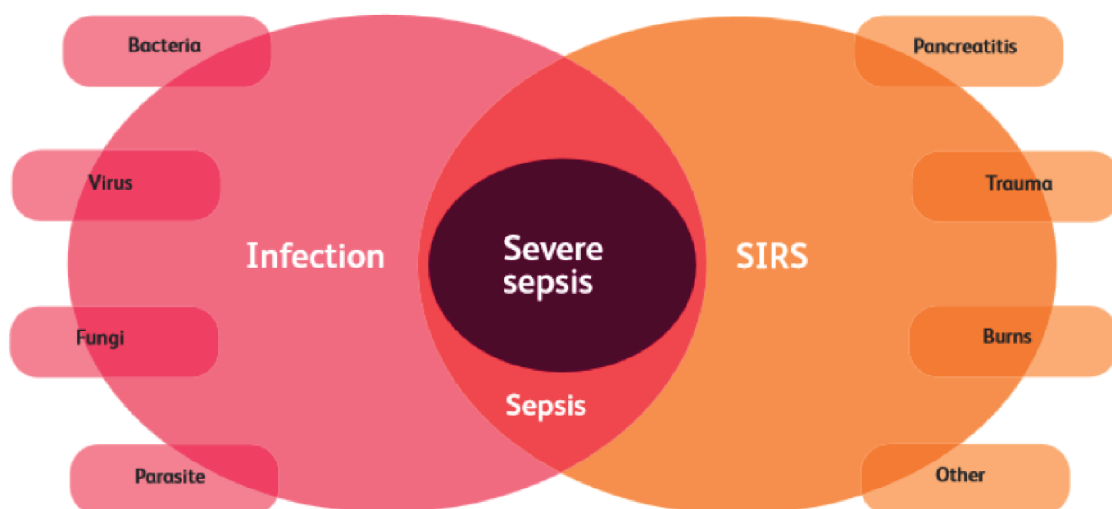
FIGURE 1 The relationship between SIRS, infection, sepsis and severe sepsis.[1] Reproduced with permission from the Royal College of Physicians.

The cost implications of sepsis are considerable. The consequences, in terms of mortality and morbidity, are severe, with Levy *et al.*[6] reporting a mortality rate of 46% for septic patients with both hypotension and a blood lactate concentration ≥ 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 than in 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 unit (ICU) 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 bloodstream 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 health-care-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 health-care-associated bloodstream infections.[12] In 2011, an estimated 6.4% [95% confidence interval (CI) 4.7% to 8.7%] of patients in acute care hospitals were diagnosed with a health-care-associated infection, with the largest proportion (23.4%) within the ICU.[13] Of patients with a health-care-associated infection, it was estimated that 7.6% had a bloodstream infection.[13] Septic shock is most commonly associated with Gram-negative bacterial bloodstream infections, but shock can also be associated with bloodstream infections caused by Gram-positive bacteria, particularly with fulminant pneumococcal, Lancefield group A streptococcal and *Staphylococcus aureus* infections.[14] Community-acquired bloodstream infections occur in people who have not had recent contact with health-care services. The spectrum of pathogens isolated from these people may differ from those associated with health-care-acquired bloodstream infection.[12]

Bloodstream infection is also a risk for people who are immunocompromised, particularly among people with neutropenia (abnormally low neutrophil levels in the blood), 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 among people who are immunocompromised.[12]

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

The types of pathogens causing bloodstream infection in children can also be slightly different from those isolated from adults with bloodstream infection. Pathogens particularly associated with community-acquired bloodstream infection in children include *Streptococcus pneumoniae*, *Neisseria meningitidis*, *S. aureus* and *Escherichia coli*. The profile of pathogens associated with health-care-associated infections in children is thought to be similar to that associated with health-care-associated infections in adults; however, polymicrobial infection and anaerobic bacteraemia are thought to occur less frequently among children.[12]

TABLE 1 The bacteria most commonly associated with bloodstream infection in adults in England, Wales and Northern Ireland between April 2011 and March 2012

Name of bacteria	Percentage of all bacteria associated with bloodstream infection	Group of bacteria
Escherichia 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	–

–, Gram negative; +, Gram positive; MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-sensitive *Staphylococcus aureus*.
Adapted from Davies[15] under the Open Government Licence (v3.0) for public sector information (www.nationalarchives. gov.uk/doc/open-government-licence/version/3/).

Diagnosis of sepsis

Diagnostic criteria for sepsis are listed in the Surviving Sepsis Campaign guidelines[16] (adapted from Levy *et al.*[17]). In summary, regular observations of all vital signs should be taken and recorded, kidney and liver function tests should be performed, and inflammatory biomarkers and serum lactate concentration 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 below). The diagnostic criteria for sepsis include the following variables:

- General variables – temperature of > 38.3 °C or < 36 °C, heart rate > 90 beats per minute, rapid breathing, altered mental status, significant oedema or high blood sugar concentration in the absence of diabetes.
- Inflammatory variables – low or high white blood cell count or more than 10% of immature forms, raised plasma C-reactive protein or raised plasma procalcitonin.
- Haemodynamic and tissue perfusion variables – low blood pressure or raised blood lactate (a concentration of ≥ 4 mmol/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 or raised plasma bilirubin levels.

Current standard of care for patients with suspected bloodstream infections or sepsis

The diagnostic work-up of sepsis and bloodstream 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*[18]
- the Royal College of Obstetricians and Gynaecologists: *Green-Top Guideline 64a. Bacterial Sepsis in Pregnancy*[19]
- the Royal College of Obstetricians and Gynaecologists: *Green-Top Guideline 64b. Bacterial Sepsis following Pregnancy*[20]
- Surviving Sepsis Campaign: *International Guidelines for Management of Severe Sepsis and Septic Shock*.[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, which is currently developing a payment framework, has announced new sepsis mandates to monitor adherence to the sepsis care pathway across the NHS.[22]

The Surviving Sepsis Campaign guidelines make the following specific recommendations relating to the detection of localised and bloodstream infection:[16]

- At least two sets of blood cultures should be collected (aerobic and anaerobic) before antimicrobial therapy is initiated, provided this does not significantly delay (> 45 minutes) the start of antimicrobial administration. At least one sample 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 fluids, such as urine, cerebrospinal fluid, wound exudate, respiratory secretions or other bodily fluids, which may be the source of infection should be obtained before initiation of antimicrobial therapy, as long as doing so does not significantly delay the start of antimicrobial administration.
- Imaging studies such as computerised tomography or radiography 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 that are indicative of sepsis:

(a) measure blood lactate levels to identify tissue hypoperfusion
(b) obtain blood cultures prior to administration of antibiotics
(c) administer broad-spectrum antibiotics
(d) administer 30 ml/kg of crystalloid for hypotension or lactate ≥ 4 mmol/l.

The 6-hour bundle should be completed within 6 hours of 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 of ≥ 65 mmHg

(f) in the event of persistent arterial hypotension despite volume resuscitation (septic shock) or initial lactate concentration of ≥ 4 mmol/l:

    – measure central venous pressure
    – measure central venous oxygen saturation

(g) remeasure lactate concentration, 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 its International Guidelines for the Management of Severe Sepsis and Septic Shock.[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 or 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.[16] Such treatment is typically referred to as 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.[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 that have previously colonised or infected the patient.

Clinicians should also consider whether or not a fungus is a likely causative pathogen when selecting initial therapy and administer, when appropriate, empirical antifungal therapy.

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 and genital tract infections
- bloodstream infections
- eye, ear, nose and throat infections
- sepsis of unknown origin.

Current practice for detecting pathogens in those with suspected bloodstream infection or sepsis

The current practice for detecting pathogens in those with suspected bloodstream 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/ionisation 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.

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.[12] A blood culture set for the diagnosis of bloodstream infection is defined as one aerobic and one anaerobic bottle.[12] For adult patients it is recommended that 20–30 ml 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.[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 (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) or BacT/ALERT (Oganon Teknika Corp., Durham, NC, USA) 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 or not there are multiple pathogens and whether or not 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 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.[12]

The first target is not typically met by laboratories because 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.[12]

Blood culture results may not detect pathogens within an individual's bloodstream because of the transient nature of bloodstream infections and a low number of organisms present in a blood sample; there can often be fewer than 1×10^3 colony-forming units (cfus) per litre in adults with a bloodstream infection.[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 in an individual's bloodstream as pathogens transferred from the skin during the drawing of blood can contaminate the culture. To reduce the incidence of such false-positive results, current standards recommended that contamination rates are no higher than 3%.[12] In addition, several criteria may be used to differentiate between contamination and true bloodstream 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 may be requested.[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.[12] The magnitude of bacteraemia is usually higher in infants and children than that in adults and, therefore, the sensitivity of detection is not believed to be significantly reduced by lower blood-to-medium ratios.[12]

Although 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]

Matrix-absorbed laser desorption/ionisation time-of-flight mass spectrometry

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 (Bruker, Billerica, MA, USA), 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. 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 with 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 randomised controlled trial (RCT) is the 28-day all-cause mortality between the two arms. Following personal communication with Dr Leeming (Dr John Leeming, North Bristol NHS Trust, 2015, personal communication), it was identified that Sepsityper had been used in the MALDI-TOF MS arm in all centres bar Newcastle upon Tyne, where the centre used its own method.

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, antifungals and antivirals) among bacteria, viruses and fungi. As existing

antimicrobial medications are becoming less effective, strategies such as the UK Five Year Antimicrobial Resistance Strategy[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 meticillin-resistant *S. aureus* infection have fallen, while there is an increase in the incidence of bloodstream infections caused by resistant Enterobacteriaceae (Gram-negative bacteria) such as *Klebsiella* spp. and *E. 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.[32] The guidance recommends that, when antimicrobials are administered empirically, the patient is reviewed after 48–72 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.[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 the treatment of 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 a 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.[32] In addition, a reduction in agents may result in cost savings.

The National Institute for Health and Care Excellence 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 RCTs had assessed the impacts of de-escalation (although only three are explicitly referenced[34–36]), four of which were set in ICUs, the exception being hospital based, and only one of which, by Leone *et al.*,[35] was in patients with sepsis. The Guideline Development Group found no evidence from these RCTs that de-escalation between 48 and 72 hours increased patient mortality. The Guideline Development Group 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, who recruited 116 patients 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%; $p = 0.03$) and in the mean number of antibiotic days (9 vs. 7.5 days; $p = 0.03$), although the increase in median duration of ICU stay (9 vs. 8 days) was not statistically significant ($p = 0.71$). The Guideline Development Group 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 in 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 by 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, the extent to which de-escalation would occur in clinical practice is unclear.

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® (Roche Diagnostics, Risch-Rotkreuz, Switzerland); SepsiTest™ (Molzym Molecular Diagnostics, Bremen, Germany); and IRIDICA BAC BSI assay (Abbott Diagnostics, Lake Forest, IL, USA)] 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 bloodstream infection, that is, the test was perfect, management strategies could be quickly altered dependent on whether or not 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. Owing 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 and 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 31.4% excess in mortality (65.7% in the inadequate group vs. 34.3% in the control group). In those without a nosocomial infection there was a significant reduction in the length of stay in ICU associated with adequate treatment (7 days in the inadequate group vs. 9 days in the control group; $p = 0.02$).

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 to 13.5 days) and excess costs were US$13,398 (95% CI US$1060 to US$26,736) when a patient had inadequate antifungal treatment. Inadequate antifungal treatment was defined as a treatment delay of ≥ 24 hours from candidaemia onset or inadequate dose of an antifungal agent active against the pathogen.

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

Morrell et al.[40] retrospectively analysed 157 consecutive patients who, over a 4-year period, developed a *Candida* bloodstream infection, of whom 50 (32%) died during hospitalisation. The number of patients in whom antifungal treatment was not delayed (> 12 hours) was nine, while treatment was delayed in 148 patients. The adjusted odds ratio associated with delay in antifungal treatment was 2.09 (95% CI 1.53 to 2.84). Delays in antifungal treatment were also associated with a longer stay in ICU (9.4 days, compared with 0.4 days for those in whom treatment was not delayed; $p = 0.019$).

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 (a pathogen that is not present is identified) and false negatives (a pathogen that is present in the blood culture is not identified). The consequences of these misdiagnoses are likely to differ. In the case of false

positives, there is the risk of overtreatment, which would incur cost and could increase the risk of antimicrobial resistance; in the case of false negatives, withdrawal of treatment could put the patient 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, therefore, that standard techniques provide an inaccurate gold standard that 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 in this report, some clinical experts believe that such results would provide valuable information in the patient treatment decision, despite adversely affecting the specificity of the test against blood culture.

Description of the technologies under assessment

Our research aims to evaluate the clinical effectiveness and cost-effectiveness of three tests that potentially allow the rapid detection and identification of bacterial and fungal deoxyribonucleic acid (DNA) in the bloodstream of patients suspected of having sepsis. These tests are the SeptiFast, SepsiTest and IRIDICA BAC BSI assays, 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 preculture steps, allowing an earlier initial assessment of the patient. It is anticipated that blood cultures and clinical judgement would be required in 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; the comparators have been described in *Current practice for detecting pathogens in those with suspected bloodstream infection or sepsis*. For brevity, where the test name alone is provided it should be assumed that this denotes its use 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 clinical judgement.

LightCycler SeptiFast Test MGRADE
The LightCycler SeptiFast Test MGRADE – henceforth referred to as SeptiFast – is a Conformité Européenne (CE)-marked in vitro diagnostic real-time polymerase chain reaction (PCR) test that simultaneously detects and identifies bacterial and fungal DNA. The test requires 1.5 ml 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 three parallel reactions (Gram-positive bacteria, Gram-negative bacteria and 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 (Roche Diagnostics, Risch-Rotkreuz, Switzerland) 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 that is intended to reduce the positive rate for coagulase-negative staphylococci and *Streptococcus* spp. based on the assumption that they are contaminants and not causal agents when the crossing point value is < 20.

Where *S. 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 meticillin resistance of *S. aureus* through PCR using the LightCycler 2.0 instrument.

The bacterial and fungal species that can be detected by SeptiFast are shown in *Table 2*.

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

Bacteria		
Gram negative	**Gram positive**	**Fungi**
E. coli	*S. aureus*	*Candida albicans*
Klebsiella (*pneumoniae/oxytoca*)	Coagulase-negative staphylococci (including *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*)	*Candida tropicalis*
Serratia marcescens	*S. pneumoniae*	*Candida parapsilosis*
Enterobacter (*cloacae/aerogenes*)	*Streptococcus* spp. (including *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus mitis*)	*Candida krusei*
Proteus mirabilis	*Enterococcus faecium*	*Candida glabrata*
Pseudomonas aeruginosa	*Enterococcus faecalis*	*Aspergillus fumigatus*
Acinetobacter baumannii		
Stenotrophomonas maltophilia		
Species often referred to as *Acinetobacter calcoaceticus–Acinetobacter baumannii* (ACB complex) are not detected.		

The test has an analytical sensitivity of 100 cfu/ml for coagulase-negative staphylococci, *Streptococcus agalactiae*, *Streptococcus pyogenes, Streptococcus pneumoniae* and *Streptococcus mitis*. The minimum analytical sensitivity for all other pathogens detected by SeptiFast is 30 cfu/ml.

SepsiTest

SepsiTest is a CE-marked PCR test for detecting bacterial and fungal DNA in 1 ml of K-EDTA- (potassium-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 three 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 it could take longer depending on laboratory workflow, and 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 bacterial or fungal species are present. Where readable sequences are available from sequence analysis, bacteria and fungi can be identified using the SepsiTest-BLAST online tool (www.sepsitest-blast.de/en/index.html). Sequencing results are typically available in 3–4 hours in optimal conditions, depending on the analyser used, equating to a 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 cfu/ml, depending on the target species.

Shortly before the submission of this report, Molzym Molecular Diagnostics, on 22 July 2015, informed NICE that it had updated SepsiTest to version 4.0 (date of change 1 July 2015). The changes reported by the company include the implementation of an internal extraction control to validate the extraction of DNA; the removal of the internal control from the kits; and the fact that processing of duplicate samples is no longer recommended. In consultation with NICE, a decision was taken to exclude the updated version of SepsiTest from the analyses described in this report, primarily because no data on the diagnostic accuracy associated with this version were provided. 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.

IRIDICA BAC BSI

The IRIDICA BAC BSI assay – henceforth referred to as IRIDICA – is a CE-marked in vitro diagnostic test for detecting and identifying bacterial and candidal DNA in 5 ml of EDTA-treated whole blood. The test can also detect the *mecA* (*Staphylococcus*-specific meticillin resistance), *vanA* and *vanB* (*Enterococcus*-specific vancomycin resistance) and *Klebsiella pneumoniae* carbapenemase-producing (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 that 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, Lake Forest, IL, USA), although the final IRIDICA system has key differences from PLEX-ID as it uses a greater volume of whole blood (5 vs. 1.5 ml, respectively) and has different desalter and mass spectrometry modules. The company supplied confidential data regarding the equivalency of IRIDICA and PLEX-ID, which the company declared demonstrated that the limits of detection of four core organisms were comparable in IRIDICA and PLEX-ID. Based on these data, the External Assessment Group was comfortable with including data from studies that used IRIDICA–PLEX-ID hybrid systems.

The IRIDICA assay is able to detect over 780 bacterial and candidal species. The mean limit of detection for the assay is 39 cfu/ml, with a range of 0.25–128 cfu/ml depending on the target species. The estimated time to result is 5 hours and 55 minutes in optimal conditions, although it may take longer depending on laboratory workflow.

The decision problem

This report aims to evaluate the clinical effectiveness and cost-effectiveness of the three interventions in comparison with blood culture, with or without MALDI-TOF MS. As detailed in *The potential benefits and possible harms of a test that could provide earlier information on pathogen*, 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 *An estimation of the time to clinically important information associated with each intervention and comparator*.

It is anticipated that good compliance with the guidelines described in *Current standard of care for patients with suspected bloodstream infections or suspected sepsis* and *Current practice for detecting pathogens in those with suspected bloodstream infection or sepsis* will be associated with better patient outcomes. As such, the potential gains associated with the three tests have likely been reduced since the introduction of the guidelines. Given the early use of broad-spectrum antibiotics, it is anticipated that there will be much greater clinical utility in accurately determining the specific pathogen causing the infection rather than in determining whether or not the patient has sepsis.

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; that is, that the test result will be reported back in a timely manner and not delayed because of 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 in 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 or fungi are present (hours) for SepsiTest[a] 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[a,b]
Interventions				
SeptiFast				6 hours
IRIDICA				6 hours
SepsiTest	4 hours (denoted as x)			(x) + 3–4 hours (range 7–8 hours)
Comparators				
Blood culture	15 hours (denoted as y) (range 12–48 hours)[c]	(y) + 12–24 hours (denoted as z)[d]	(z)	(z) + 12–18 hours[e] (range 36–90 hours)
Blood culture with MALDI-TOF MS	(y)	(z)	(z)	(z) (range 24–72 hours)
Blood culture with MALDI-TOF MS and Sepsityper	(y)	(y) + 1–13 hours[d]	(z)	(z) (range 24–72 hours)

a　Assuming optimal workflow conditions for the interventions. These times may be extended depending on work patterns and location of the required equipment.
b　Note that a subsequent identification based on blood culture methods will also become available.
c　Based on the time at which a blood culture bottle flags positive.
d　Positive blood cultures have been subcultured on agar plates. The time taken is dependent on the time of day at which blood culture positivity is reported.
e　The time taken is based on the speed of bacterial growth.
Note
Shading denotes that these components are not reported individually, but the combined information is provided by the next non-shaded column.

Chapter 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 for undertaking systematic reviews[41] and the Cochrane Diagnostic Test Accuracy Working Group on the meta-analysis of diagnostic tests.[42,43]

This report contains reference to confidential information provided as part of the NICE appraisal process. This information has been removed from the report and the results, discussions and conclusions of the report do not include the confidential information. These sections are clearly marked in the report.

Methods for reviewing effectiveness

Identification of studies

Electronic databases
Studies were identified by searching the following electronic databases and research registers from January 2006 to May 2015:

- MEDLINE In-Process & Other Non-Indexed Citations and MEDLINE (via OvidSP)
- EMBASE (via OvidSP)
- Cochrane Database of Systematic Reviews (via Wiley Online Library)
- Cochrane Central Register of Controlled Trials (via Wiley Online Library)
- Health Technology Assessment Database (via Wiley Online Library)
- Database of Abstracts of Review of Effects (via Wiley Online Library)
- Science Citation Index Expanded (via the Web of Science)
- Conference Proceedings Index-Science (via the Web of Science)
- World Health Organization's International Clinical Trials Registry Platform
- Current Controlled Trials
- National Institutes of Health ClinicalTrials.gov
- Manufacturer and User Facility Device.

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 (i.e. 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.*[44] in 2006 with subsequent full-text peer-reviewed publications by Mancini *et al.*[45] and Louie *et al.*[46] in 2008. 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.*[47] in 2008, with a subsequent full-text peer-reviewed publication by Wellinghausen *et al.*[48] in 2009. 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.*,[49] in 2014, who 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 16 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 animal-only studies was introduced). An example of the MEDLINE search strategy is provided in *Appendix 1*.

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 were undertaken using the Google search engine (Google Inc., Mountain View, CA, USA), 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 ResearchSoft, San Francisco, CA, USA).

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 studies for inclusion ($n = 87$) were then adjudicated by three clinical experts independently (GDP, 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 criteria below.

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 of which the 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.

Population

All studies of adults and children (of any age) with suspected bloodstream 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.*,[3] which found that a significant number of poor outcomes from severe systemic infection occurs in the absence of SIRS criteria at inception.

Target conditions

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

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 the IRIDICA assay).

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, these would also form comparators for each intervention.

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, readmission 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.

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. When 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 were extracted from the systematic review in the first instance, but were cross-checked for accuracy with the original papers. When necessary, additional data were extracted from the original papers. For the review of SepsiTest and IRIDICA, all data were extracted from the original papers. Unpublished study data from the company (which were 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).

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.[51] [Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2)]. Further details are provided in *Appendix 2*.

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 pairwise meta-analysis.

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 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:

$$TP_i \sim \text{Binomial}(\pi_{Ai}, (TP_i + FN_i)). \tag{1}$$

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:

$$TP_i \sim \text{Binomial}(\pi_{Bi}, (FP_i + TN_i)). \tag{2}$$

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

$$\mu_{Ai} = \text{logit}(\pi_{Ai}) \tag{3}$$

$$\mu_{Bi} = \text{logit}(\pi_{Bi}). \tag{4}$$

Sensitivity and specificity are correlated within each 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, $(\mu_A, \mu_B)^\top$, respectively, and variance–covariance matrix, Σ_{AB}, such that:

$$\begin{pmatrix} \mu_{Ai} \\ \mu_{Bi} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu_A \\ \mu_B \end{pmatrix}, \Sigma_{AB} \right) \tag{5}$$

$$\Sigma_{AB} = \begin{pmatrix} \sigma_A^2 & \sigma_{AB} \\ \sigma_{AB} & \sigma_B^2 \end{pmatrix}. \tag{6}$$

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:

$$\mu_A \sim N(0, 10000) \tag{7}$$

$$\mu_B \sim N(0, 10000) \tag{8}$$

$$\Sigma_{AB} \sim \text{IW}\left(\begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}, \upsilon = 2 \right). \tag{9}$$

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

This prior distribution has a between-study standard deviation (SD) of 1.5 [95% credible interval (CrI) 0.4 to 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:

$$\Sigma_{AB} \sim \text{IW}\left(\begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}, \upsilon = 5 \right). \tag{10}$$

This prior distribution has a between-study SD of 0.5 (95% CrI 0.3 to 1.4).

Reasons for the heterogeneity in sensitivity and specificity between studies were explored using metaregression. Models with and without covariates were compared using the deviance information criterion, 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 (version 1.4.3; MRC Biostatistics Unit, Cambridge, UK).[54] Analyses were conducted in R using the R2WinBUGS interface package (version 3.1.2; R Foundation for Statistical Computing, Vienna, Austria).[55] Convergence was assessed using the Gelman–Rubin convergence statistic. Convergence was achieved relatively quickly and generally within 5000 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 plots with 95% CrIs and 95% prediction intervals for sensitivity and specificity.

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, readmission rate and change in antimicrobial treatment plan) and clinical outcome measures (e.g. 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[56] and the Centre for Reviews and Dissemination for undertaking systematic reviews,[41,57] a narrative synthesis of included studies (grouped by outcome) was undertaken.

Clinical effectiveness results

Quantity and quality of research available

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*.

Number and type of studies excluded

A total of 111 full-text articles were excluded, as they did not meet all the prespecified inclusion criteria. The majority of the articles were excluded primarily on the basis of having insufficient information to allow calculation of a diagnostic 2 × 2 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*.
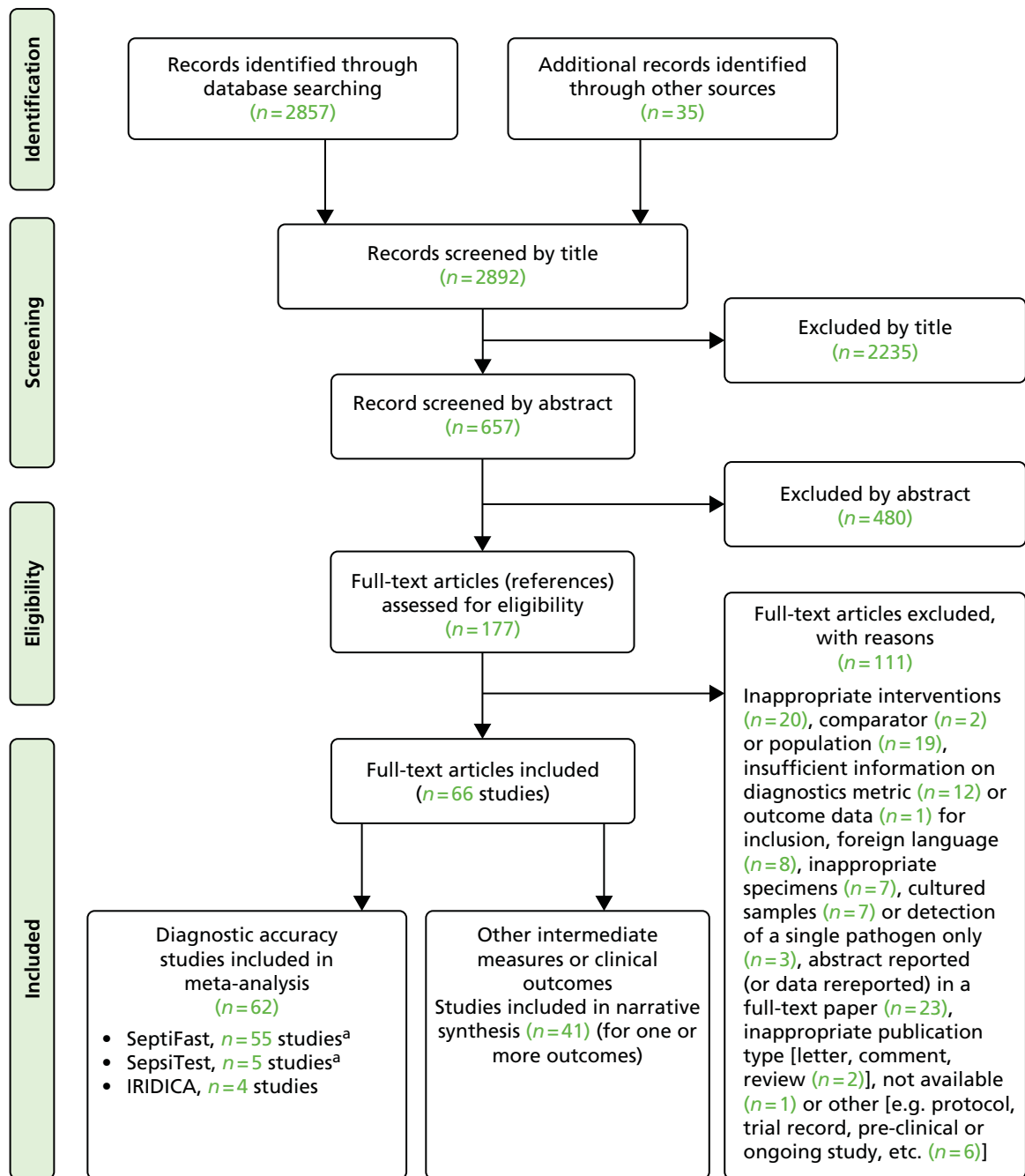
FIGURE 2 Study flow chart (adapted from Moher *et al.*[58]): clinical effectiveness review. a, Two studies included both SeptiFast and SepsiTest and are counted as individual studies in each test comparison (meta-analysis) with the reference standard.

Assessment of effectiveness

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,[44–46,59–111] and one study[112] 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,[100,110,112] all SeptiFast studies were diagnostic cohort studies. Two single index test studies[48,113] compared SepsiTest with blood culture, and one study evaluated SepsiTest with blood culture and MALDI-TOF MS.[114] Two three-arm studies[118,119] compared both SeptiFast and SepsiTest with blood culture. Four single index test studies compared IRIDICA with blood culture,[49,115–117] two of which employed IRIDICA–PLEX-ID hybrid systems[49,116] (confidential 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[46,80] and one IRIDICA study[49] were conducted in North America. One IRIDICA study did not report the country.[115] Two SeptiFast studies[100,107] were conducted in Brazil, two were undertaken in Japan[82,87] and one was undertaken in Turkey.[105] Two SeptiFast studies[63,111] were undertaken in the UK. (Confidential information has been removed.)

Twenty-four of the SeptiFast studies,[46,65,67,71,74–77,81,82,84,85,90,95,99–101,104–107,110–112] one multitest SeptiFast and SepsiTest study,[119] (confidential information has been removed) had data collected prospectively.

Eight of the SeptiFast studies[64,66,69,86,89,96,97,109] and one SepsiTest study[114] had a retrospective design. One SeptiFast study[103] evaluated samples collected both retrospectively and prospectively. (Confidential information has been removed.)

Where reported, the sampling period ranged from 2 months[45] to 66 months.[80]

The clinical setting (e.g. community, emergency department, hospital, intensive/critical care, general/specialist) was not reported for nine SeptiFast studies,[44,65,69,72,88,98,100,105,110] or for one multitest SeptiFast and SepsiTest study[118] (confidential information has been removed). The setting across the remaining studies varied (see *Table 4*). Explicit inclusion and exclusion criteria for patients were reported for seven SeptiFast studies,[74,80,83,106,110–112] (confidential information has been removed) and one IRIDICA study.[116]

With the exception of the SeptiFast study by Warhurst *et al.*,[111] which reported on health-care-associated bloodstream infections, and the SeptiFast study by Josefson *et al.*,[85] which reported on community-acquired bloodstream infections, reporting of whether infection was community or hospital acquired was unclear in the remainder of the included studies.

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,[44,59–63,65,68–70,72,73,79,81,82,84,86,88,93,98,99,101,102,108] one SepsiTest study,[48] and one multitest SeptiFast and SepsiTest study[118] (confidential information has been removed) did not report on the mean or median age of patients. Six of the SeptiFast studies[70,85,93,96,99,111] included both adults and children, two included children and neonates[86,109] and one included children and infants.[105] Three SeptiFast studies[69,73,98] and one IRIDICA study[115] included neonates and infants, and one SepsiTest study included adults and children.[48] (Confidential information has been removed.)

TABLE 4 Study characteristics of included studies (chronologically ordered by intervention)

Single index test studies: SeptiFast compared with blood culture

Author (year)	Country	Clinical setting	Study design[a]	Total number of patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Raglio et al. (2006)[44] (abstract)	NR	NR	Single gate, NR	74 (114)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Bingold et al. (2007)[59] (abstract)	Germany	Intensive/critical care	Single gate, NR	21 (134)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Klemm et al. (2007)[60] (abstract)	Germany	Intensive/critical care	Single gate, NR	44 (56)	Test accuracy (patient), other intermediary/clinical outcomes	NR
Lodes et al. (2008)[61] (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)[62] (correspondence)	Croatia	In hospital and intensive/ critical care	Single gate, NR	36 (39)	Test accuracy (sample)	NR
Dark et al. (2009)[63] (correspondence)	UK	Intensive/critical care	Single gate, NR	50 (90)	Test accuracy (pathogen)	NR
Dierkes et al. (2009)[64]	Germany	Intensive/critical care	Single gate, retrospective	77 (99)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics (partly)
Gimeno et al. (2009)[65] (abstract)	Spain	NR	Single gate, prospective	19 (45)	Test accuracy (sample)	NR
Lehmann et al. (2009)[66]	Germany	Emergency department, in hospital and intensive/critical care	Single gate, retrospective	436 (NR)	Intermediary/clinical outcomes	Roche Diagnostics (partly)
Lodes et al. (2009)[67]	Germany	Intensive/critical care	Single gate, prospective	52 (258)	Test accuracy (sample)	NR
Palomares et al. (2009)[68] (abstract)	Spain	Intensive/critical care	Single gate, NR	73 (76)	Test accuracy (sample), other intermediary/clinical outcomes	NR

Author (year)	Country	Clinical setting	Study design[a]	Total number of patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Paolucci et al. (2009)[69] (correspondence)	Italy	NR	Single gate, retrospective	34 (NR)	Test accuracy (patient), other intermediary/clinical outcomes	NR
Varani et al. (2009)[70]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	100 (130)	Test accuracy (episode)	NR
von Lilienfeld-Toal et al. (2009)[71]	Germany	In hospital	Single gate, prospective	70 (784)	Test accuracy (pathogen)	Roche Diagnostics (partly)
Westh et al. (2009)[72]	Germany	NR	Single gate, NR	359 (558)	Test accuracy (pathogen), other intermediary/clinical outcomes	Roche Diagnostics
Berger et al. (2010)[73] (abstract)	Austria	Neonatal unit	Single gate, NR	38 (38)	Test accuracy (patient)	NR
Bloos et al. (2010)[74]	Germany and France	Intensive/critical care	Single gate, prospective	142 (236)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics
Lamoth et al. (2010)[75]	Switzerland	In hospital	Single gate, prospective	86 (237)	Test accuracy (episode)	Roche Diagnostics
Lehmann et al. (2010)[76]	Germany	Intensive/critical care	Single gate, prospective	108 (453)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics
Maubon et al. (2010)[77]	France	In hospital and unclear if intensive/critical care	Single gate, prospective	110 (110)	Test accuracy (patient), other intermediary/clinical outcomes	Roche Diagnostics
Regueiro et al. (2010)[78]	Spain	In hospital and intensive/critical care	Single gate, NR	72 (106)	Test accuracy (sample), other intermediary/clinical outcomes	No
Soki et al. (2010)[79] (abstract)	Hungary	In hospital and intensive/critical care	Single gate, NR	159 (162)	Test accuracy (sample)	NR
Tsalik et al. (2010)[80]	USA	Emergency department	Single gate, NR	306 (306)	Test accuracy (patient) other intermediary/clinical outcomes	No
Wallet et al. (2010)[81]	France	Intensive/critical care	Single gate, prospective	72 (102)	Test accuracy (pathogen) other intermediary/clinical outcomes	Roche Diagnostics (partly)
Yanagihara et al. (2010)[82]	Japan	In hospital and emergency department	Single gate, prospective	212 (400)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Diagnostics

TABLE 4 Study characteristics of included studies (chronologically ordered by intervention) (*continued*)

Author (year)	Country	Clinical setting	Study design[a]	Total number of patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Bravo et al. (2011)[83]	Spain	In hospital and intensive/critical care	Single gate, NR	53 (53)	Test accuracy (episode)	NR
Hettwer et al. (2011)[84]	Germany	Emergency department	Single gate, prospective	113 (113)	Test accuracy (patient)	Roche Diagnostics
Josefson et al. (2011)[85]	Sweden	In hospital	Single gate, prospective	1093 (1141)	Test accuracy (patient), other intermediary/clinical outcomes	Roche Diagnostics (partly)
Lucignano et al. (2011)[86]	Italy	In hospital and intensive/critical care	Single gate, retrospective	803 (1553)	Test accuracy (sample)	NR
Obara et al. (2011)[87]	Japan	Emergency department, in hospital and intensive/critical care	Single gate, NR	54 (78)	Test accuracy (sample)	Roche Diagnostics (partly)
Vrioni et al. (2011)[88] (abstract)	Greece	NR	Single gate, NR	33 (33)	Test accuracy (patient), other intermediary/clinical outcomes	NR
Alvarez et al. (2012)[89]	Spain	Intensive/critical care	Single gate, retrospective	102 (NR)	Intermediary/clinical outcomes	NR
Grif et al. (2012)[90]	Austria	In hospital and intensive/critical care	Single gate, prospective	61 (71)	Test accuracy (sample), other intermediary/clinical outcomes	Pfizer
Guido et al. (2012)[91]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	166 (166)	Test accuracy (sample)	NR
Lodes et al. (2012)[92]	Germany	Intensive/critical care	Single gate, NR	104 (148)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Mauro et al. (2012)[93]	Italy	In hospital and unclear if intensive/critical care	Single gate, NR	79 (79)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Pasqualini et al. (2012)[94]	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)[95]	Germany	Intensive/critical care	Single gate, prospective	170 (225)	Test accuracy (sample)	NR
Tschiedel et al. (2012)[96]	Germany	In hospital and intensive/critical care	Single gate, retrospective	75 (110)	Test accuracy (sample) other intermediary/clinical outcomes	NR
Herne et al. (2013)[97]	Estonia	In hospital and intensive/critical care	Single gate, retrospective	144 (160)	Test accuracy (sample), other intermediary/clinical outcomes	NR

Author (year)	Country	Clinical setting	Study design[a]	Total number of patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Kasper and Altiok (2013)[98]	Austria	NR	Single gate, NR	46 (NR)	Test accuracy (patient)	Roche Diagnostics (partly)
Paolucci et al. (2013)[99]	Italy	In hospital	Single gate, prospective	201 (437)	Test accuracy (episode), other intermediary/clinical outcomes	No
Rodrigues et al. (2013)[100] (abstract)	Brazil	NR	Single-gate, prospective RCT	46 (NR)	Intermediary/clinical outcomes	NR
Avolio et al. (2014)[101]	Italy	Emergency department and intensive/critical care	Single gate, prospective	525 (525)	Test accuracy (pathogen), other intermediary/clinical outcomes	NR
Burdino et al. (2014)[102]	Italy	In hospital and intensive/critical care	Single gate, NR	1024 (1186)	Test accuracy (sample)	NR
Mancini et al. (2014)[103]	Italy	In hospital	Single gate, retrospective and prospective data	228 (NR)	Intermediary/clinical outcomes	Roche Diagnostics
Markota et al. (2014)[104]	Slovenia	Intensive/critical care	Single gate, prospective	57 (63)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Ozkaya-Parlakay et al. (2014)[105]	Turkey	NR	Single gate, prospective	69 (79)	Test accuracy (sample), other intermediary/clinical outcomes	NR
Schaub et al. (2014)[106]	Switzerland	Emergency department	Single gate, prospective	110 (205)	Test accuracy (patient), other intermediary/clinical outcomes	Roche Diagnostics (partly)
Sitnik et al. (2014)[107]	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)[108] (abstract)	Italy	In hospital	Single gate, NR	491 (1837)	Test accuracy (sample)	NR
Calitri et al. (2015)[109]	Italy	In hospital and intensive/critical care	Single gate, retrospective	289 (NR)	Test accuracy (episode)	No
Idelevich et al. (2015)[110]	Germany	NR	Single-gate, prospective RCT	150 (253)	Test accuracy (pathogen), other intermediary/clinical outcomes	Roche Diagnostics and Pfizer (partly)
Warhurst et al. (2015)[111]	UK	Intensive/critical care	Single gate, prospective	795 (NR)	Test accuracy (pathogen) other intermediary/clinical outcomes	No

TABLE 4 Study characteristics of included studies (chronologically ordered by intervention) (*continued*)

Author (year)	Country	Clinical setting	Study design[a]	Total number of patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Single index test studies: SeptiFast compared with blood culture plus MALDI-TOF MS						
Tafelski et al. (2015)[112]	Germany	Intensive/critical care	Single-gate, prospective RCT	78 (78)	Test accuracy (sample), other intermediary/clinical outcomes	Roche Deutschland GmbH
Single index test studies: SepsiTest compared with blood culture						
Wellinghausen et al. (2009)[48]	Germany	Intensive/critical care	Single gate, prospective	187 (342)	Test accuracy (sample), other intermediary/clinical outcomes	No
Nieman et al. (2015)[113]	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed
Single index test studies: SepsiTest compared with blood culture plus MALDI-TOF MS						
Loonen et al. (2014)[114]	The Netherlands	Emergency department	Single gate, retrospective	125 (NR)	Test accuracy (sample), other intermediary/clinical outcomes	Molzym GmbH (partly)
Single index test studies: IRIDICA compared with blood culture						
Bacconi et al. (2014)[49]	USA	Emergency department	Single gate, prospective	331 (331)	Test accuracy (sample)	NR but majority of authors are employees of Ibis Biosciences (an Abbott company)
Delco-Volante et al. (2015)[115] (conference presentation)	NR	NR	Single gate, prospective	NR (81)	Test accuracy (sample)	Abbott
Vincent et al. (2015)[116]	Belgium, UK, Switzerland, France, Poland and Germany	Intensive/critical care	Single gate, prospective	529 (NR)	Test accuracy (sample), other intermediary/clinical outcomes	Ibis Biosciences, Inc., Abbott
Metzgar et al. (2015)[117]	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed

Two index test studies: SeptiFast and SepsiTest compared with blood culture

Author (year)	Country	Clinical setting	Study design[a]	Total number of patients (paired blood tests)	Outcomes (unit of analysis)	Commercially funded
Leitner et al. (2013)[118]	Austria	NR	Single gate, NR	57 (75)	Test accuracy (sample)	No
Schreiber and Nierhaus (2013)[119]	Germany	Intensive/critical care	Single gate, prospective	50 (NR)	Test accuracy (patient), other intermediary/clinical outcomes	Molzym GmbH and Roche Diagnostics (partly)

NR, not reported.

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 or 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).

TABLE 5 Patient characteristics of included studies (chronologically ordered by intervention)

Author (year)	Population	Mean age, years (median)	% male	% suspected community-/hospital-acquired infection	% immunocompromised patients	% antibiotics at time of blood sample collection
Single index test studies: SeptiFast (without MALDI-TOF)						
Raglio et al. (2006)[44] (abstract)	Patients with suspected sepsis	NR	NR	NR	NR	NR
Bingold et al. (2007)[59] (abstract)	Surgical patients with severe sepsis and septic shock	NR	NR	NR	NR	NR
Klemm et al. (2007)[60] (abstract)	Patients in intensive care with suspected sepsis	NR	NR	NR	NR	NR
Lodes et al. (2008)[61] (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)[62] (correspondence)	Patients with suspected sepsis	NR	NR	NR	NR	100
Dark et al. (2009)[63] (correspondence)	Adults with suspected sepsis	NR	NR	NR	NR	NR
Dierkes et al. (2009)[64]	Adults with suspected sepsis	(55)	63.6	NR	45	79.2
Gimeno et al. (2009)[65]	Patients (oncohaematological) with febrile neutropenia	NR	NR	NR	NR	100
Lehmann et al. (2009)[66]	Adults with suspected sepsis	54.8	61.5	NR	NR	NR
Lodes et al. (2009)[67]	Adults with suspected sepsis	60.5	57.7	NR	NR	NR
Palomares et al. (2009)[68] (abstract)	Adults with suspected sepsis	NR	NR	NR	NR	93.2
Paolucci et al. (2009)[69] (correspondence)	Neonates with suspected sepsis	NR	NR	NR	NR	NR

Author (year)	Population	Mean age, years (median)	% male	% suspected community-/hospital-acquired infection	% immunocompromised patients	% antibiotics at time of blood sample collection
Varani et al. (2009)[70]	Adults and children (immunocompromised) with suspected sepsis	NR	NR	NR	100	100
von Lilienfeld-Toal et al. (2009)[71]	Adults (haematological) with febrile neutropenia	(60)	54	NR	NR	0
Westh et al. (2009)[72]	Patients with suspected sepsis	NR	NR	NR	NR	NR
Berger et al. (2010)[73] (abstract)	Very low birthweight infants (neonates) with suspected sepsis	NR	NR	NR	NR	NR
Bloos et al. (2010)[74]	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)[75]	Adults (haematological) with febrile neutropenia	(54)	62	NR	NR	NR
Lehmann et al. (2010)[76]	Adults with suspected sepsis	58.4	66.7	NR	NR	NR
Maubon et al. (2010)[77]	Patients with malignancies and suspected sepsis	56.3	60.9	NR	NR	88.2
Regueiro et al. (2010)[78]	Adults with suspected sepsis	64	73.6	NR	NR	NR
Soki et al. (2010)[79] (abstract)	Septic patients in intensive care or with haematological malignancies	NR	NR	NR	NR	NR
Tsalik et al. (2010)[80]	Adults with suspected sepsis	54.1	54.9	NR	NR	22.5
Wallet et al. (2010)[81]	Adults with suspected sepsis	NR	NR	NR	NR	NR
Yanagihara et al. (2010)[82]	Patients with suspected sepsis	NR	64.6	NR	NR	NR
Bravo et al. (2011)[83]	Adult ICU patients who were critically ill with suspected sepsis	(65.5)	62.3	NR	NR	0

TABLE 5 Patient characteristics of included studies (chronologically ordered by intervention) (*continued*)

Author (year)	Population	Mean age, years (median)	% male	% suspected community-/hospital-acquired infection	% immunocompromised patients	% antibiotics at time of blood sample collection
Hettwer et al. (2011)[84]	Adults with suspected sepsis	NR	NR	NR	NR	NR
Josefson et al. (2011)[85]	Adults and children with suspected sepsis	(67)	55.5	100 (community acquired)	NR	NR
Lucignano et al. (2011)[86]	Neonates and children with suspected sepsis	NR	NR	NR	NR	NR
Obara et al. (2011)[87]	Adults with suspected sepsis	61.6	64.8	NR	NR	NR
Vrioni et al. (2011)[88] (abstract)	Patients with suspected sepsis	NR	NR	NR	NR	NR
Alvarez et al. (2012)[89]	Adults with severe sepsis or septic shock	64.9	78.4	NR	NR	NR
Grif et al. (2012)[90]	Adults with suspected sepsis	55.6	68.9	NR	NR	91.8
Guido et al. (2012)[91]	Adult neutropenic patients (with haematological malignancies) with suspected sepsis	(66.1)	62	NR	NR	0
Lodes et al. (2012)[92]	Adults with suspected sepsis	63.1	71.1	NR	NR	79.7% of samples under antibiotic therapy and 41.9% under antifungal
Mauro et al. (2012)[93]	Adults and children, immunocompromised with suspected sepsis	NR	51.9	NR	100	5
Pasqualini et al. (2012)[94]	Adults with suspected sepsis	(73)	55	NR	4.3	48.8
Rath et al. (2012)[95]	Adults (who have undergone liver transplantation or other major abdominal surgery) with suspected sepsis	56.4	56.5	NR	NR	NR

Author (year)	Population	Mean age, years (median)	% male	% suspected community-/hospital-acquired infection	% immunocompromised patients	% antibiotics at time of blood sample collection
Tschiedel et al. (2012)[96]	Adults and children with suspected sepsis	(6)	49.3	NR	NR	NR
Herne et al. (2013)[97]	Adults with suspected sepsis	58	42.4	NR	NR	99.3
Kasper and Altiok (2013)[98]	Very low-birthweight premature infants with suspected sepsis	NR	NR	NR	NR	0
Paolucci et al. (2013)[99]	Adults and children (haematological) with severe febrile neutropenia	NR	NR	NR	NR	0
Rodrigues et al. (2013)[100] (abstract)	Adults with suspected sepsis	64.5	67.4	NR	NR	0
Avolio et al. (2014)[101]	Adults with suspected sepsis	NR	NR	NR	NR	NR
Burdino et al. (2014)[102]	Adults with suspected sepsis	NR	NR	NR	10.5	89
Mancini et al. (2014)[103]	Adults (haematological patients) with suspected sepsis	48.6	66.7	NR	NR	NR
Markota et al. (2014)[104]	Adults with severe sepsis or septic shock	59.5	66.7	NR	NR	61.9
Ozkaya-Parlakay et al. (2014)[105]	Children with severe sepsis or septic shock	2.7	62.3	NR	0	NR
Schaub et al. (2014)[106]	Adults with suspected sepsis	(64)	51	NR	13.6	14.5
Sitnik et al. (2014)[107]	Adults with suspected sepsis	49.7	64.9	NR	NR	NR
Barbanti et al. (2015)[108]	Patients (haematological) with febrile neutropenia and suspected sepsis	NR	NR	NR	NR	NR

TABLE 5 Patient characteristics of included studies (chronologically ordered by intervention) (*continued*)

Author (year)	Population	Mean age, years (median)	% male	% suspected community-/hospital-acquired infection	% immunocompromised patients	% antibiotics at time of blood sample collection
Calitri *et al.* (2015)[109]	Children and neonates with suspected sepsis, febrile neutropenia, fever without focus or localised infective focus	(6.8)	63.3	NR	NR	NR
Idelevich *et al.* (2015)[110]	Adults with febrile neutropenia or afebrile neutropenia with sepsis	52.4	59.3	NR	NR	100
Warhurst *et al.* (2015)[111]	Adults (over 16 years) with suspected sepsis	(58)	60	100 (health-care acquired)	NR	85.7
*Single index test studies: **SeptiFast (with MALDI-TOF)***						
Tafelski *et al.* (2015)[112]	Adults with suspected sepsis	(63, average median)	64.1	NR	15.4	NR
*Single index test studies: **SepsiTest (without MALDI-TOF)***						
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
Nieman *et al.* (2015)[113]	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed
*Single index test studies: **SepsiTest (with MALDI-TOF)***						
Loonen *et al.* (2014)[114]	Adults with suspected sepsis	64.7	59.2	NR	NR	NR

Author (year)	Population	Mean age, years (median)	% male	% suspected community-/hospital-acquired infection	% immunocompromised patients	% antibiotics at time of blood sample collection
Single index test studies: IRIDICA						
Bacconi et al. (2014)[49]	Adults with suspected sepsis	NR	NR	NR	NR	NR
Delco-Volante et al. (2015)[115] (conference presentation)	Neonates with suspected sepsis	(0)	NR	NR	NR	0
Vincent et al. (2015)[116]	Adults with suspected or proven sepsis or severe infection	61	64.6	NR	16.6	NR
Metzgar et al. (2015)[117]	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed
Two index test studies: SeptiFast and SepsiTest						
Leitner et al. (2013)[118]	Critically ill patients with suspected sepsis	NR	NR	NR	NR	NR
Schreiber and Nierhaus (2013)[119]	Adults with suspected sepsis	(64)	80	NR	NR	72

NR, not reported.

Twenty-two SeptiFast studies,[44,45,62,63,65,68,70–72,75,78,79,81,83,85,88,96,98–100,105,108] one multitest SeptiFast and SepsiTest study,[118] (confidential information has been removed) did not report details or a reference to a guideline for defining 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 (see *Appendix 4*).

Ten SeptiFast studies[45,46,64,70,93,94,102,105,106,112] and one IRIDICA study[116] reported on the proportion of the included patients who were immunocompromised. In addition, Paolucci *et al.*[69] 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[62,64,65,68,70,71,77,80,83,90–94,97–100,102,104,106,110,111] reported on the proportion of patients receiving antimicrobial therapy at the time of blood sampling. In addition, it was unclear in one SeptiFast study[74] if patients received antimicrobial therapy (98%) prior to blood sampling. Of the 23 SeptiFast studies, six[71,83,91,98–100] reported that none of the included patients had received antimicrobial therapy at the time of blood sampling. Similarly, in one IRIDICA study[115] none of the included patients received antimicrobial therapy at the time of blood sampling. In one multitest SeptiFast and SepsiTest study,[119] the majority of patients (72%) received antimicrobial therapy at recruitment. (Confidential information has been removed.)

The SeptiFast studies by Alvarez *et al.*,[89] Bloos *et al.*,[74] Bingold *et al.*[59] and Markota *et al.*[104] reported that all included participants had severe sepsis or septic shock. Bloos *et al.*[74] also reported a mean SOFA score of 10 and SAPS II score of 49 for the entire cohort. Markota *et al.*[104] reported a mean admission APACHE score for the cohort of 25 (SD ± 7.6). The SeptiFast studies by Herne *et al.*[97] and Lehmann *et al.*[76] reported that all included patients had severe sepsis. Seven SeptiFast studies[46,77,80,83,100,106,112] and the one multitest SeptiFast and SepsiTest study[119] reported mixed samples of patients with sepsis, severe sepsis and septic shock in varying proportions. The SeptiFast RCT by Rodrigues *et al.*[100] reported a mean APACHE II score of 17 for the SeptiFast group and 17 for the blood culture group, but was unclear whether or not this was at study entry or following testing. The SeptiFast RCT by Tafelski *et al.*[112] reported a median SAPS II on admission for the SeptiFast group of 40 (interquartile range 32–50) and the blood culture group of 47 (interquartile range 34–65). Schreiber and Nierhaus[119] also reported a median SAPS II score of 41 (interquartile range 33 to 49) for the entire cohort. The IRIDICA study by Vincent *et al.*[116] reported a mean SOFA score at baseline of 7.6 (SD 4.2) indicating a 15–20% risk in mortality in ICU, but did not report proportions of the patients with sepsis, severe sepsis or septic shock. The remainder of the included studies did not report on the proportion of patients with sepsis, severe sepsis or septic shock, or disease severity.

Across the included studies, the number of patients analysed ranged from 19 (45 paired blood samples) (SeptiFast – Gimeno *et al.*[65]) to 1093 (1114 paired blood samples) (SeptiFast – Josefson *et al.*[85]).

Details of index and reference tests, blood sampling methods and Conformité Européenne 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.[45,69,71–78,80–83,85–87,90–94,96–99,101–103,107,109–112,114] Of these, nine studies reported blood volumes that did not comply with CE approval: Lehmann *et al.*,[76] Lodes *et al.*[92] and von Lilienfeld-Toal *et al.*[71] all reported using 1 ml in adults; Bloos *et al.*,[74] Lamoth *et al.*,[75] Paolucci *et al.*[99] and Sitnik *et al.*[107] all reported using 3 ml in adults; Berger *et al.*[73] reported using 0.1 ml in neonates and infants; and Kasper and Altiok[98] reported using 0.1–0.7 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.[45,60,64,65,68,70–72,74–76,78,81–87,90–99,101,102,104–107,110–112] Of these, one SeptiFast study reported that blood drawn for SeptiFast and for blood culture were drawn within 1 hour,[102] and another study reported that blood drawn for SeptiFast and for blood culture were drawn within 12 hours of each other.[97] 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.*,[66] Westh *et al.*,[72] Tsalik *et al.*,[80] Wallet *et al.*[81] and Yanagihara *et al.*[82] 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, 19 reported using the BACTEC system.[60,64,70,71,75,76,79,83,85–87,92–96,107,110,112] Of these, one used the BACTEC system with MALDI-TOF MS.[112] Seventeen studies reported using the BacT/ALERT system.[45,78,84,90,91,97–104,106,108,109,111] 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 13 studies evaluating SeptiFast.[45,64,76,90,96,97,99,101–104,110,112] Working times were 7 days per week for four SeptiFast studies,[45,64,97,101] 6 days per week for one SeptiFast study[104] and 5 days per week for six SeptiFast studies.[90,96,99,103,110,112] 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 12 SeptiFast studies.[74,76,83,85,86,94,97,101,106,112,118,119] Definition of a true positive varied across these studies (*Table 6*).

A range of metrics (units of analyses) was used to assess the diagnostic accuracy of SeptiFast. The unit of analyses was 'patients' in 11 studies,[46,60,69,73,77,80,84,85,88,98,106] pathogens in seven studies[63,71,72,81,101,110,111] and episodes in five studies.[70,75,83,99,109] For the remainder of the studies evaluating SeptiFast against blood culture (with or without MALDI-TOF MS) the unit of analysis was samples. Although 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.[44,45,64,67–70,73,76,78,81–85,87,91–98,105–107,109,110,120] and eight studies reported that contaminants were excluded.[46,72,80,86,99,101,102,111] For the remainder of the SeptiFast studies it was unclear if contaminants were included or excluded.

(Confidential information has been removed.) One study of SepsiTest did not report when bloods were drawn.[114] One study performed blood culture using a BACTEC system,[48] (confidential information has been removed) and one study reported using BacT/ALERT with MALDI-TOF MS.[114] (Confidential information has been removed.) Definition of a true positive was reported by one SepsiTest study.[48] (Confidential information has been removed.)

(Confidential information has been removed.) The study by Delco-Volante *et al.*[115] reported using 0.5 ml of blood in neonates and infants. (Confidential information has been removed.)

Neither of the studies evaluating both SeptiFast and SepsiTest against blood culture reported the volume of blood used for the index test assay.[118,119] Leitner *et al.*[118] reported that the reference standard and index tests were performed on blood samples drawn at the same time. Schreiber and Nierhaus[119] 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.*[118] was samples and the unit of analysis for Schreiber and Nierhaus[119] was patients. Both studies included contaminants in the diagnostic test accuracy analysis.

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)[44] (abstract)	U	U	U	U	U	U	U
Bingold et al. (2007)[59] (abstract)	U	U	U	U	U	U	U
Klemm et al. (2007)[60] (abstract)	U	U	U	U	U	U	U
Lodes et al. (2008)[61] (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)[62] (correspondence)	U	U	U	U	U	U	U
Dark et al. (2009)[63] (correspondence)	U	L	U	U	N	U	U
Dierkes et al. (2009)[64]	U	U	U	U	Y	U	U
Gimeno et al. (2009)[65] (abstract)	U	U	U	U	U	U	U
Lehmann et al. (2009)[66]	U	U	U	H	N	N	U
Lodes et al. (2009)[67]	U	U	U	U	U	U	U
Palomares et al. (2009)[68] (abstract)	U	U	U	U	U	U	U
Paolucci et al. (2009)[69] (correspondence)	U	U	U	U	N	N	U
Varani et al. (2009)[70]	U	U	U	L	N	U	U
Von Lilienfeld-Toal et al. (2009)[71]	U	U	U	U	U	Y	U
Wellinghausen et al. (2009)[48]	U	U	U	L	U	N	U
Westh et al. (2009)[72]	U	U	U	H	U	N	U
Berger et al. (2010)[73] (abstract)	U	U	U	U	U	Y	U

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Bloos et al. (2010)[74]	U	L	U	U	N	Y	U
Lamoth et al. (2010)[75]	U	U	U	U	U	Y	U
Lehmann et al. (2010)[76]	U	U	U	L	N	Y	U
Maubon et al. (2010)[77]	U	U	U	U	N	N	U
Regueiro et al. (2010)[78]	U	U	U	L	N	N	U
Soki et al. (2010)[79] (abstract)	U	U	U	U	U	U	U
Tsalik et al. (2010)[80]	U	U	U	H	N	N	U
Wallet et al. (2010)[81]	U	U	U	H	U	N	U
Yanagihara et al. (2010)[82]	U	U	U	H	N	N	U
Bravo et al. (2011)[83]	H	L	U	U	Y	N	U
Hettwer et al. (2011)[84]	U	U	U	H	N	U	U
Josefson et al. (2011)[85]	U	U	U	H	U	N	U
Lucignano et al. (2011)[86]	U	U	U	H	U	N	U
Obara et al. (2011)[87]	U	U	U	L	N	N	U
Vrioni et al. (2011)[88] (abstract)	U	U	U	U	U	U	U
Alvarez et al. (2012)[89]	U	H	U	U	N	U	U
Grif et al. (2012)[90]	U	U	U	L	N	N	U
Guido et al. (2012)[91]	U	U	U	L	U	N	U
Lodes et al. (2012)[92]	U	U	U	L	N	Y	U
Mauro et al. (2012)[93]	U	U	U	L	N	N	U
Pasqualini et al. (2012)[94]	U	U	U	L	U	N	U
Rath et al. (2012)[95]	U	U	U	L	Y	U	U

TABLE 6 Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study (*continued*)

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Tschiedel et al. (2012)[96]	U	U	U	L	U	N	U
Herne et al. (2013)[97]	U	U	U	H	N	N	U
Kasper and Altiok (2013)[98]	U	U	U	L	U	Y	U
Leitner et al. (2013)[118]	U	U	U	U	U	U	U
Paolucci et al. (2013)[99]	U	U	U	U	U	Y	U
Rodrigues et al. (2013)[100] (abstract)	U	U	U	U	U	U	U
Schreiber and Nierhaus (2013)[119]	U	L	U	U	N	U	U
Avolio et al. (2014)[101]	U	U	U	H	Y	N	U
Bacconi et al. (2014)[49]	U	U	U	U	U	N	U
Burdino et al. (2014)[102]	U	U	U	L	U	N	U
Loonen et al. (2014)[114]	U	U	U	H	U	N	U
Mancini et al. (2014)[103]	U	U	U	U	N	N	U
Markota et al. (2014)[104]	U	U	U	U	N	U	U
Ozkaya-Parlakay et al. (2014)[105]	U	U	U	U	N	U	U
Schaub et al. (2014)[106]	U	U	U	U	N	U	U
Sitnik et al. (2014)[107]	U	U	U	U	N	Y	U
Barbanti et al. (2015)[108]	U	U	U	U	U	U	U
Calitri et al. (2015)[109]	U	U	U	U	Y	N	U
Delco-Volante et al. (2015)[115] (conference presentation)	U	U	U	U	U	Y	U
Idelevich et al. (2015)[110]	U	U	U	U	U	N	U

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Tafelski et al. (2015)[112]	H	L	L	L	N	N	U
Warhurst et al. (2015)[111]	L	L	L	L	N	N	U
Vincent et al. (2015)[116]	N	N	U	U	N	N	U
Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed
Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed

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.

Quality characteristics

The QUADAS-2 tool,[51] designed to evaluate the methodological quality of diagnostic accuracy studies, comprises four key domains: 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. With the exception of Warhurst *et al.*,[111] all other studies were considered to be at risk of bias and to have concerns regarding applicability.[121]

(Confidential information has been removed.)

(Confidential information has been removed.)

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 *Meta-analysis* have been presented.

Diagnostic test accuracy

A total of 62 studies contributed to the meta-analysis of sensitivity and specificity, including two studies[118,119] that were three-arm (two index tests) studies. For simplicity, the correlation between tests was ignored in the analyses.

In total, 54 studies[44–46,59–65,67–88,90–99,101,102,104–111,118,119] evaluated SeptiFast compared with blood culture, four studies[48,113,118,119] evaluated SepsiTest compared with blood culture and four studies[49,115–117] evaluated IRIDICA compared with blood culture. Separate meta-analyses are presented for each of these three tests in *SeptiFast test*, *SepsiTest* and *IRIDICA assay compared with blood culture*. In addition, one study[112] evaluated SeptiFast compared with blood culture plus MALDI-TOF MS and one study[114] evaluated SepsiTest compared with blood culture plus MALDI-TOF MS. As there was only one study for each of these comparisons, no meta-analysis was conducted and the data were summarised narratively.

SeptiFast test *SeptiFast test compared with blood culture* The pooled sensitivity and specificity of SeptiFast compared with blood culture (54 studies) were 0.65 (95% CrI 0.60 to 0.71) and 0.86 (95% CrI 0.84 to 0.89), respectively (*Figure 4*). The 95% prediction intervals of 0.29 to 0.90 (sensitivity) and 0.62 to 0.96 (specificity) suggest considerable uncertainty in predicting the sensitivity and specificity of a new study. The between-study SDs for logit sensitivity and specificity were estimated to be 0.76 (95% CrI 0.57 to 1.01) and 0.66 (95% CrI 0.53 to 0.85), respectively, with a correlation of –0.05 (95% CrI –0.38 to 0.28). *Figure 5* presents the joint distribution for sensitivity and specificity. 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 a median of 17%.
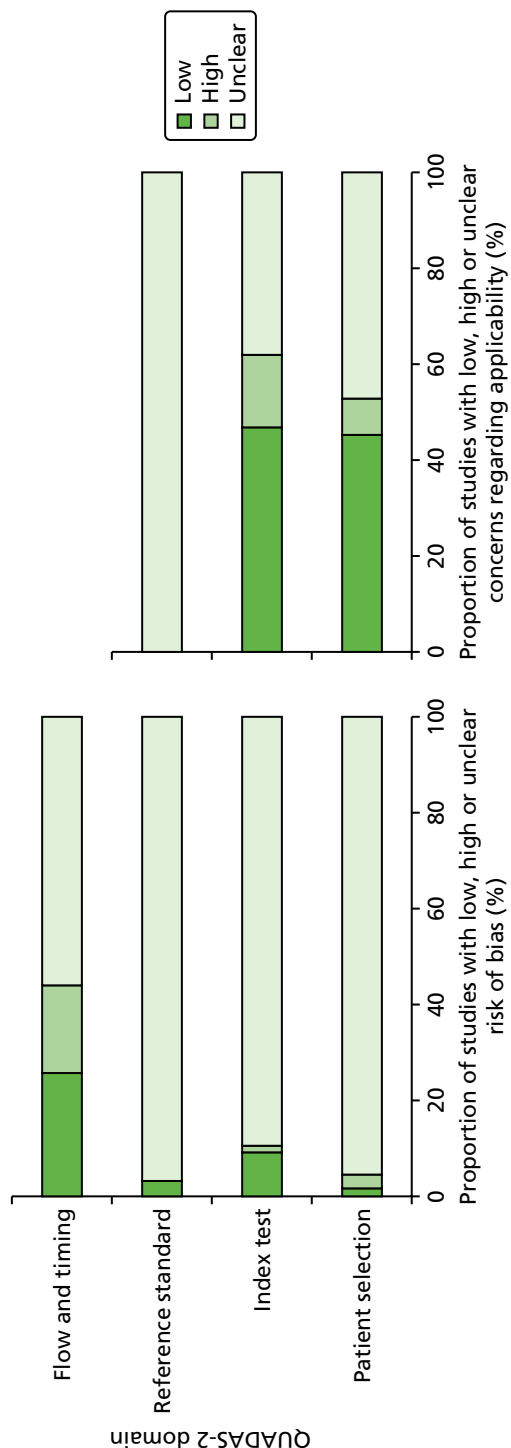
FIGURE 3 Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies.

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)[44]	✓						
Bingold *et al.* (2007)[59]	✓						
Klemm *et al.* (2007)[60]	✓						
Louie *et al.* (2008)[46]	✓						
Mancini *et al.* (2008)[45]	✓						
Dierkes *et al.* (2009)[64]	✓		✓	✓	✓		✓
Lehmann *et al.* (2009)[66]				✓	✓		✓
Palomares *et al.* (2009)[68]	✓						
Paolucci *et al.* (2009)[69]	✓						
Westh *et al.* (2009)[72]			✓				
Bloos *et al.* (2010)[74]				✓	✓		
Lehmann *et al.* (2010)[76]					✓		
Maubon *et al.* (2010)[77]	✓						✓
Regueiro *et al.* (2010)[78]				✓			
Tsalik *et al.* (2010)[80]	✓			✓	✓		
Wallet *et al.* (2010)[81]	✓						✓
Hettwer *et al.* (2011)[84]			✓				
Josefson *et al.* (2011)[85]	✓			✓			
Vrioni *et al.* (2011)[88]	✓						✓
Alvarez *et al.* (2012)[89]				✓	✓		
Grif *et al.* (2012)[90]				✓			✓
Lodes *et al.* (2012)[92]				✓			✓

TABLE 7 Summary of intermediate and clinical outcomes reported across studies (*continued*)

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
Mauro *et al.* (2012)[93]	✓						
Pasqualini *et al.* (2012)[94]				✓			
Tschiedel *et al.* (2012)[96]	✓						✓
Herne *et al.* (2013)[97]	✓						✓
Paolucci *et al.* (2013)[99]			✓				
Rodrigues *et al.* (2013)[100]		✓		✓	✓		✓
Avolio *et al.* (2014)[101]	✓						
Mancini *et al.* (2014)[103]				✓	✓		✓
Markota *et al.* (2014)[104]				✓	✓		✓
Ozkaya-Parlakay *et al.* (2014)[105]				✓	✓		
Schaub *et al.* (2014)[106]	✓		✓		✓		
Sitnik *et al.* (2014)[107]	✓						
Idelevich *et al.* (2015)[110]	✓	✓		✓	✓		✓
Tafelski *et al.* (2015)[112]	✓	✓	✓	✓	✓	✓	✓
Warhurst *et al.* (2015)[111]			✓	✓	✓		
Single index test studies: SepsiTest							
Loonen *et al.* (2014)[114]				✓			
Single index test studies: IRIDICA							
Confidential information has been removed		Confidential information has been removed					
Vincent *et al.* (2015)[116]				✓			✓
Two index test studies: SeptiFast and SepsiTest							
Schreiber and Nierhaus (2013)[119]				✓			

Study	TP	FP	FN	TN	Sensitivity (95% CrI)		Specificity (95% CrI)	
Raglio (2006)[44]	13	24	4	73	0.73 (0.54 to 0.88)		0.77 (0.68 to 0.84)	
Bingold (2007)[59]	10	52	10	62	0.55 (0.36 to 0.73)		0.58 (0.49 to 0.66)	
Klemm (2007)[60]	13	5	8	30	0.63 (0.45 to 0.79)		0.86 (0.75 to 0.93)	
Lodes (2008)[61]	25	41	21	271	0.56 (0.42 to 0.69)		0.87 (0.83 to 0.90)	
Louie (2008)[46]	28	17	15	140	0.65 (0.52 to 0.77)		0.89 (0.84 to 0.93)	
Mancini (2008)[45]	20	14	1	68	0.86 (0.71 to 0.95)		0.84 (0.76 to 0.90)	
Vince (2008)[62]	4	9	4	22	0.58 (0.33 to 0.80)		0.76 (0.62 to 0.87)	
Dark (2009)[63]	9	9	2	73	0.75 (0.53 to 0.90)		0.89 (0.82 to 0.94)	
Dierkes (2009)[64]	14	13	9	63	0.62 (0.45 to 0.78)		0.84 (0.75 to 0.90)	
Gimeno (2009)[65]	2	6	0	37	0.72 (0.42 to 0.92)		0.86 (0.76 to 0.93)	
Lodes (2009)[67]	12	21	20	205	0.43 (0.28 to 0.58)		0.90 (0.86 to 0.94)	
Palomares (2009)[68]	7	10	3	56	0.68 (0.45 to 0.86)		0.85 (0.77 to 0.92)	
Paolucci (2009)[69]	3	4	1	26	0.69 (0.39 to 0.89)		0.87 (0.75 to 0.94)	
Varani (2009)[70]	22	10	16	82	0.59 (0.45 to 0.73)		0.89 (0.82 to 0.94)	
von Lilienfeld-Toal (2009)[71]	11	18	32	73	0.31 (0.20 to 0.46)		0.82 (0.73 to 0.88)	
Westh (2009)[72]	50	124	24	382	0.68 (0.57 to 0.77)		0.76 (0.72 to 0.79)	
Berger (2010)[73]	17	9	0	12	0.88 (0.72 to 0.96)		0.69 (0.50 to 0.83)	
Bloos (2010)[74]	31	51	8	146	0.77 (0.64 to 0.87)		0.75 (0.69 to 0.81)	
Lamoth (2010)[75]	9	26	26	80	0.33 (0.20 to 0.48)		0.77 (0.69 to 0.84)	
Lehmann (2010)[76]	40	74	18	321	0.69 (0.57 to 0.79)		0.82 (0.78 to 0.85)	
Maubon (2010)[77]	17	11	15	67	0.56 (0.40 to 0.70)		0.86 (0.78 to 0.92)	
Regueiro (2010)[78]	17	1	10	78	0.63 (0.47 to 0.78)		0.95 (0.90 to 0.98)	
Soki (2010)[79]	21	18	18	102	0.56 (0.41 to 0.70)		0.85 (0.79 to 0.91)	
Tsalik (2010)[80]	40	13	26	227	0.61 (0.50 to 0.71)		0.94 (0.91 to 0.96)	
Wallet (2010)[81]	4	11	6	81	0.51 (0.27 to 0.73)		0.88 (0.81 to 0.93)	
Yanagihara (2010)[82]	22	23	10	345	0.68 (0.53 to 0.81)		0.93 (0.91 to 0.95)	
Bravo (2011)[83]	12	4	13	24	0.52 (0.35 to 0.69)		0.86 (0.74 to 0.94)	
Hattwar (2011)[84]	39	5	12	57	0.75 (0.63 to 0.85)		0.91 (0.83 to 0.95)	
Joseison (2011)[85]	50	64	86	896	0.38 (0.31 to 0.46)		0.93 (0.92 to 0.95)	
Lucignano (2011)[86]	136	90	24	1303	0.84 (0.78 to 0.89)		0.93 (0.92 to 0.95)	
Obara (2011)[87]	11	10	1	56	0.81 (0.60 to 0.93)		0.85 (0.77 to 0.92)	
Vrioni (2011)[88]	7	1	1	24	0.76 (0.52 to 0.92)		0.91 (0.81 to 0.97)	
Grif (2012)[90]	5	12	3	51	0.65 (0.39 to 0.85)		0.82 (0.73 to 0.89)	
Guido (2012)[91]	21	17	2	126	0.84 (0.69 to 0.93)		0.88 (0.82 to 0.92)	
Lodes (2012)[92]	25	34	5	84	0.80 (0.65 to 0.90)		0.73 (0.65 to 0.80)	
Mauro (2012)[93]	27	5	7	42	0.80 (0.67 to 0.90)		0.88 (0.79 to 0.95)	
Pasqualini (2012)[94]	32	28	25	306	0.57 (0.45 to 0.69)		0.91 (0.88 to 0.94)	
Rath (2012)[95]	57	35	23	110	0.71 (0.61 to 0.79)		0.77 (0.70 to 0.83)	
Tschiedel (2012)[96]	12	14	7	77	0.64 (0.45 to 0.81)		0.85 (0.77 to 0.91)	
Herne (2013)[97]	30	41	12	99	0.71 (0.57 to 0.82)		0.72 (0.65 to 0.79)	
Kasper (2013)[98]	15	9	0	22	0.87 (0.70 to 0.96)		0.76 (0.62 to 0.87)	
Leitner (2013)[118]	3	8	4	60	0.55 (0.29 to 0.78)		0.88 (0.80 to 0.93)	
Paolucci (2013)[99]	49	22	34	159	0.60 (0.49 to 0.69)		0.88 (0.83 to 0.92)	
Schreiber (2013)[119]	3	4	10	33	0.38 (0.19 to 0.60)		0.89 (0.78 to 0.95)	
Avolio (2014)[101]	83	40	56	356	0.60 (0.52 to 0.68)		0.90 (0.87 to 0.92)	
Burdino (2014)[102]	131	75	50	893	0.72 (0.65 to 0.78)		0.92 (0.90 to 0.94)	
Markota (2014)[103]	7	4	1	51	0.76 (0.52 to 0.92)		0.91 (0.83 to 0.96)	
Ozkaya-Parlakay (2014)[105]	18	1	5	55	0.75 (0.58 to 0.87)		0.94 (0.88 to 0.98)	
Schaub (2014)[106]	16	5	11	78	0.60 (0.44 to 0.76)		0.92 (0.87 to 0.96)	
Sitnik (2014)[107]	12	10	4	88	0.72 (0.52 to 0.87)		0.89 (0.83 to 0.94)	
Barbanti (2015)[108]	174	346	144	1173	0.55 (0.50 to 0.60)		0.77 (0.75 to 0.79)	
Calitri (2015)[109]	39	29	43	434	0.49 (0.39 to 0.59)		0.93 (0.91 to 0.95)	
Idelevich (2015)[110]	38	66	37	141	0.52 (0.42 to 0.63)		0.69 (0.63 to 0.75)	
Warhurst (2015)[111]	44	123	44	745	0.51 (0.41 to 0.61)		0.86 (0.83 to 0.88)	
Pooled estimate					0.65 (0.60 to 0.71)		0.86 (0.84 to 0.89)	
95% PrI					(0.29 to 0.90)		(0.62 to 0.96)	

0.9   0.95   1   0.5   0.75   1

FIGURE 4 Sensitivity and specificity of SeptiFast compared with blood culture. FN, false negative; FP, false positive; PrI, prediction interval; TN, true negative; TP, true positive.
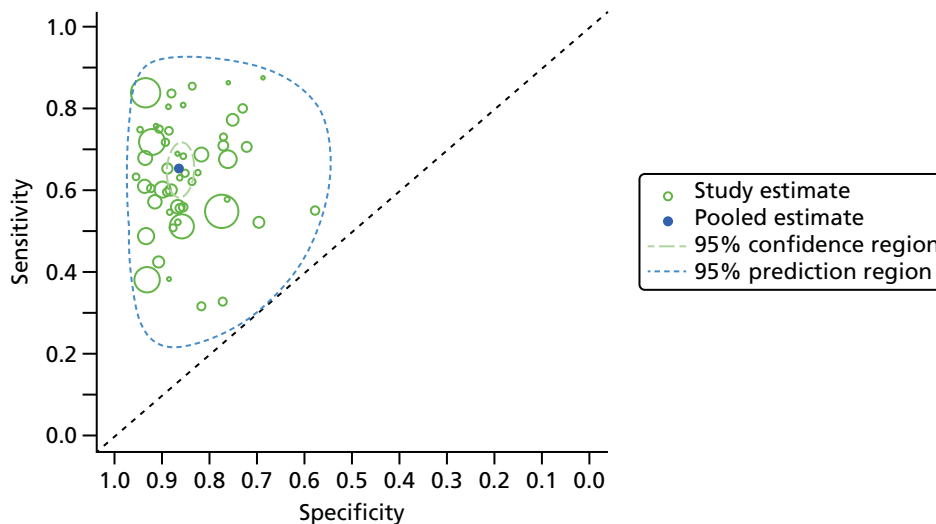
FIGURE 5 Summary receiver operating curve plot of SeptiFast compared with blood culture (all studies). The 95% prediction interval indicates the extent of heterogeneity between studies. The green circles represent the sensitivity and specificity estimates from each study, with the circle size reflecting the study sample size.

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 *Quality characteristics*) and people who were immunocompromised to allow a meaningful estimate of test accuracy. None of the subgroups analysed was shown to significantly affect the results.

- *Neonates and children* Six studies provided data on children and neonates. Of these, three studies included neonates only,[69,73,98] one included children only aged 1 month to 17 years[105] and two included both neonates and children.[86,109] Of the remaining studies, six were conducted in adults and children,[70,85,93,96,99,111] 28 were conducted in adults[45,46,63,64,67,68,71,74–76,78,80,81,83,84,87,90–92,94,95,97,101,102,104,106,110,119] and 14 did not report the age of participants.[44,59–62,65,72,77,79,82,88,107,108,118] 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 (see *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[24,62,64,65,68,70,71,74,77,80,83,90–94,97–99,104,106,110,111,119] and ranged from 0% to 100% with a median of 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 (see *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[45,59–64,67,68,70,71,73–79,81,83,85,86,90–97,99,102,104,107–109,111,119]), emergency department (three studies[80,84,106]), mixed setting of emergency or other hospital department (four studies[46,82,87,101]) or not recorded (nine studies[44,65,69,72,88,98,105,110,118]). Based on comparison of models with and without covariates for the clinical setting, there was no evidence that this affected sensitivity and specificity (see *Appendix 5*).
- *People with febrile neutropenia* In total, eight studies provided data on patients with febrile neutropenia.[45,65,71,75,91,99,108,110] Of these, six studies included patients (100%) with febrile neutropenia only.[65,71,75,91,99,108] Studies by Mancini *et al.*[45] and Idelevich *et al.*[110] reported that 92% and 98% of patients had febrile neutropenia, respectively. Based on a 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 (see *Appendix 5*).

- *Studies with inclusion/exclusion of contaminants* In total, 32 studies[44,45,64,67–70,73,76,78,81–85,87,91–98,105–110,118,119] included contaminants in the reported results, eight studies[46,72,80,86,99,101,102,111] excluded contaminants and 14 did not report on handling of contaminants.[59–63,65,71,74,75,77,79,88,90,104] Based on a comparison of models with and without covariates for the inclusion/exclusion of contaminants, there was no evidence that this affected sensitivity and specificity (see *Appendix 5*).

SeptiFast test compared with blood culture plus MALDI-TOF MS Only one study,[112] 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 to 0.86) and 0.74 (95% CI 0.64 to 0.85), respectively.

SepsiTest *SepsiTest compared with blood culture* The pooled sensitivity and specificity of SepsiTest compared with blood culture was 0.48 (95% CrI 0.21 to 0.74) and 0.86 (95% CrI 0.78 to 0.92), respectively (*Figure 6*). The 95% prediction intervals of 0.07 to 0.90 (sensitivity) and 0.66 to 0.95 (specificity) suggest considerable uncertainty in predicting the sensitivity and specificity of a new study. The between-study SDs for logit sensitivity and specificity were estimated to be 0.90 (95% CrI 0.50 to 1.92) and 0.45 (95% CrI 0.27 to 0.90), with a correlation of –0.03 (95% CrI –0.73 to 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). Owing to insufficient information provided in the included studies, planned subgroup analyses were not conducted and there were insufficient studies to conduct meaningful analyses.

SepsiTest compared with blood culture plus MALDI-TOF MS Only one study[114] that 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 to 0.23) and 0.96 (95% CI 0.92 to 1.00), respectively.

IRIDICA assay compared with blood culture The pooled sensitivity and specificity of IRIDICA compared with blood culture was 0.81 (95% CrI 0.69 to 0.90) and 0.84 (95% CrI 0.71 to 0.92), respectively (*Figure 8*). The 95% prediction intervals of 0.55 to 0.94 (sensitivity) and 0.50 to 0.96 (specificity) suggest considerable uncertainty in predicting the sensitivity and specificity of a new study. The between-study SDs for logit sensitivity and specificity were estimated to be 0.46 (95% CrI 0.28 to 0.93) and 0.65 (95% CrI 0.39 to 1.27), with a correlation of 0.06 (95% CrI –0.71 to 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). Owing 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 Confidential information has been removed.

FIGURE 7 Confidential information has been removed.

FIGURE 8 Confidential information has been removed.

FIGURE 9 Confidential information has been removed.

Other intermediate measures and clinical outcomes

A total of 41 studies provided data on one or more intermediate and/or clinical outcome measures: 37 SeptiFast studies,[44–46,59,60,64,66,68,69,72,74,76–78,80,81,84,85,88–90,92–94,96,97,99–101,103–107,110–112] one SepsiTest study,[114] two IRIDICA studies[116,117] and one study evaluating both SeptiFast and SepsiTest.[119] 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, for example 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 readmission 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.

Time to result (pathogen identification)　A summary of the studies reporting the times to pathogen identification of the index and reference test is presented in *Table 8*. Twenty-one SeptiFast studies reported data on the time to availability of results/pathogen identification.[44–46,59,60,64,68,69,77,80,81,85,88,93,96,97,101,106,107,110,112] 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 4 hours[68] to a median of 26.25 hours [range 6.75–79 hours (for samples collected at beginning of a weekend)].[64] 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. Although the majority of studies that reported data on time to pathogen identification suggest that results are obtained sooner using SeptiFast than using blood culture, laboratory working times (e.g. laboratories operating on weekdays only) might delay the time to pathogen identification in real clinical practice. Time to pathogen identification was not reported by any of the studies evaluating SepsiTest or IRIDICA.

Time to treatment　Time to treatment was reported by three SeptiFast studies, one of which was the RCT by Tafelski *et al.*[112] comparing SeptiFast with blood culture and MALDI-TOF MS, and two of which were the RCTs by Rodrigues *et al.*[100] and Idelevich *et al.*[110] comparing SeptiFast with blood culture. The time to treatment modification reported by all three RCTs was shorter in the SeptiFast group than in the blood culture group. Tafelski *et al.*[112] reported that the mean (SD) time from initially drawing blood to adaptation of empirical antimicrobial treatment was 18.8 (SD 5.6) hours in the intervention group (SeptiFast, $n = 4$), compared with 38.3 (SD 14.5) hours in the control group ($n = 5$) (*p*-value for difference not reported). The number of patients with therapy modification based on a positive diagnostic test was 4 out of 41 (9.8%) in the SeptiFast group and 5 out of 37 (13.5%) in the blood culture group. Rodrigues *et al.*[100] reported a mean time to change in therapy of 580 minutes (9.7 hours) with SeptiFast, compared with 3007 minutes (50.1 hours) (*p*-value for the between-group difference = 0.004) for blood culture. The number of patients in whom an adjustment of treatment was performed was 6 out of 17 (35%) in the SeptiFast group and 7 out of 29 (24%) in the blood culture group. Idelevich *et al.*[110] reported that the median time to the first change to a targeted antimicrobial therapy was significantly shorter with SeptiFast (21.4 hours, range 16.2–46.3 hours) than with blood culture (47.5 hours, range 7.3–59.2 hours) (*p*-value for the between-group difference = 0.018). There were 14 and 12 changes in antimicrobial therapy as a result of microbiological findings in the study group and the control group, respectively.

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 (non-comparative)		
Raglio et al. (2006)[44]	16–30 hours	5–7 days
Bingold et al. (2007)[59]	6 hours	24–48 hours
Klemm et al. (2007)[60]	6.5 hours (minimum)	2 days
Louie et al. (2008)[46]	6.54 hours (mean)	65 hours (median) (range 24–214 hours)
Mancini et al. (2008)[45]	NR	Detection with blood culture (lowest to highest range of mean number of hours): 10 hours for E.coli to 22.2 hours for coagulase-negative staphylococci. Definitive identification (lowest to highest range of mean number of hours): 44.2 hours for coagulase-negative staphylococci to 56.6 hours for E. faecalis
Dierkes et al. (2009)[64]	18 hours (median): twice daily analysis (range 6.75–74 hours for samples collected at beginning of weekend). 26.25 hours (median): once-daily analysis (range 6.75–79 hours for samples collected at beginning of weekend)	NR
Palomares et al. (2009)[68]	4 hours	6.5 hours
Paolucci et al. (2009)[69]	Information on antimicrobial susceptibility or microorganism viability ≈ 8 hours	48–72 hours
Maubon et al. (2010)[77]	6.5 hours	NR
Tsalik et al. (2010)[80]	6.5 hours (approximately)	NR
Wallet et al. (2010)[81]	7–15 hours	24–72 hours
Josefson et al. (2011)[85]	6 hours	NR
Vrioni et al. (2011)[88]	7–15 hours	24–72 hours
Mauro et al. (2012)[93]	6 hours (approximately)	NR
Tschiedel et al. (2012)[96]	17 hours (range 6–17 hours)	48 hours (range 48–120 hours, median 120 hours)
Herne et al. (2013)[97]	NR (range 5–22 hours)	NR
Avolio et al. (2014)[101]	Mean 16.6 hours (95% CI 14.9 to 18.2 hours) or median 15 hours (range 13–17 hours) (excludes SeptiFast and blood culture negative results)	Mean 84.2 hours (95% CI 82 to 86.4 hours) or median 80 hours (range 79–84 hours) (excludes SeptiFast and blood culture-negative results)
Schaub et al. (2014)[106]	6 hours	Median 16 hours (range 6–44 hours)
Sitnik et al. (2014)[107]	< 8 hours (mean)	3.5 days (mean) for blood culture-positive results. 5 days for blood culture-negative results
SeptiFast studies (comparative: RCTs)		
Idelevich et al. (2015)[110]	20.3 hours (mean)	58.3 hours (mean)
Tafelski et al. (2015)[112]	Mean 15.9 hours (SD ± 5.9 hours) (95% CI 14.1 to 17.7 hours, assuming $n = 41$)[a]	Mean 38.1 hours (SD ± 11.6 hours) (95% CI 34.4 to 41.8 hours, assuming $n = 37$)[a]

NR, not reported.
a Estimated by authors.

Test failure rates (internal control, reagents, other) Seven SeptiFast studies[64,72,84,99,106,111,112] reported information relating to failure rates. In the SeptiFast studies, test failure rates ranged from 1.5%[106] to 24.2%.[84] A summary of the failure rates reported by the SeptiFast studies is presented in *Table 9*. No data on failure rates associated with SepsiTest were available.

(Confidential information has been removed.)

The clinical significance of failure rates is that no additional information will be provided by the tests. As such, it is expected that the patient will be treated as would be the case without the test being available.

Duration of ICU and/or hospital stay Thirteen of the included studies,[64,74,76,80,89,100,103–106,110–112] all of which evaluated SeptiFast compared with blood culture, reported details of ICU and/or hospital stay. Details of these studies and the length of stay are reported in *Table 10*. Across the RCTs, Idelevich *et al.*[110] reported no statistically significant between-group difference in either ICU or hospital length of stay ($p = 0.815$ and $p = 0.235$, respectively); Tafelski *et al.*[112] also reported no significant between-group differences in either ICU or hospital length of stay ($p \geq 0.05$ for both comparisons); and Rodrigues *et al.*[100] reported no statistically significant between-group difference in hospital stay ($p = 0.632$). 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.

Change in antimicrobial treatment plan Details of change in antimicrobial treatment plan were reported by 14 SeptiFast studies[64,66,77,81,88,90,92,96,97,100,103,104,110,112] and one IRIDICA study.[116] 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.[64,77,81,88,90,92,96,97,104] These studies did not report on changes based on blood culture results. One SeptiFast study reported on changes based on the blood results only.[66] The SeptiFast RCT by Rodrigues *et al.*[100] reported that 6 out of 17 (35%) patients in the SeptiFast group and 7 out of 29 (21%) patients in the blood culture group had an adjustment of antimicrobial therapy. The corresponding numbers for the RCT by Idelevich *et al.*[110] were 7 out of 74 (9.5%) patients in the SeptiFast group and 8 out of 76 (10.5%) patients in the blood culture group. The RCT by Tafelski *et al.*[112] reported that 4 out of 41 (9.8%) patients in the SeptiFast and blood culture with MALDI-TOF MS group and 5 out of 37 (13.5%) patients in the blood culture with MALDI-TOF MS group had an adjustment of antimicrobial therapy. A *p*-value for the between-group difference was not reported by any of these RCTs.

TABLE 9 Test failure rates (internal control, reagents, other)

Author (year)	Reported test failure rate details
SeptiFast studies	
Dierkes *et al.* (2009)[64]	One failure was attributed to technical problems during the analysis; however, no further details were provided
Westh *et al.* (2009)[72]	70/558 (12.5% of episodes)
Hettwer *et al.* (2011)[84]	38/157 (24.2%)
Paolucci *et al.* (2013)[99]	100/437 (22.9% of samples corresponding to 75 febrile episodes)
Schaub *et al.* (2014)[106]	3/205 (1.5% of samples had technical failure where the internal control was not detected)
Tafelski *et al.* (2015)[112]	4/37 (10.8%)
Warhurst *et al.* (2015)[111]	69/1006 (6.9% of episodes) [SeptiFast assay failure: reagent control ($n = 6$), internal control ($n = 56$) and other reasons ($n = 7$)]

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 (non-comparative)	
Dierkes et al. (2009)[64]	Hospital stay: 35 days (median)
Bloos et al. (2010)[74]	ICU stay: 13 days (median)
	Hospital stay: 34 days (median)
Lehmann et al. (2010)[76]	ICU stay true negatives: 17 days (range 1–89 days)
	ICU stay true positives: 36 days (range 8–87 days)
	Hospital stay true negatives: 23 days (range 1–93 days)
	Hospital stay true negatives: 38 days (range 8–90 days)
Tsalik et al. (2010)[80]	Hospital stay: 6.3 days (mean)
Alvarez et al. (2012)[89]	ICU stay, SeptiFast: 22.9 days (mean) (SD ± 29.9 days)
	ICU stay, blood culture: 31.0 days (mean) (SD ± 19.4 days)
	Hospital stay, SeptiFast: 18.3 days (mean) (SD ± 21.4 days)
	Hospital stay, blood culture: 21.3 days (mean) (SD ± 23.4 days)
	Between-group difference ICU and hospital: $p < 0.05$
Mancini et al. (2014)[103]	Hospital stay: no between-group differences were observed (no data reported)
Markota et al. (2014)[104]	Hospital stay: 27 days (mean) (SD ± 28.9 days)
Ozkaya-Parlakay et al. (2014)[105]	ICU stay: 15.3 days (mean) (SD ± 23.8 days)
Schaub et al. (2014)[106]	Hospital stay: 11 days (median)
Warhurst et al. (2015)[111]	ICU stay: 16 days (IQR 9–30 days)
SeptiFast studies (comparative: RCTs)	
Rodrigues et al. (2013)[100]	Hospital stay, SeptiFast: 32 days (mean)
	Hospital stay, blood culture: 31 days (mean)
	Between-group difference: $p = 0.632$
Idelevich et al. (2015)[110]	ICU stay, SeptiFast: 0.8 days (mean) (SD ± 4.0 days)
	ICU stay, blood culture: 0.9 days (mean) (SD ± 3.4 days)
	Hospital stay, SeptiFast: 40.4 days (mean) (SD ± 25.3 days)
	Hospital stay, blood culture: 42.9 days (mean) (SD ± 22.0 days)
	Between-group difference: hospital, $p = 0.235$; ICU, $p = 0.815$
Tafelski et al. (2015)[112]	ICU stay, SeptiFast: 34 days (range 13–65 days)
	ICU stay, blood culture: 32 days (range 16–57 days)
	Hospital stay, SeptiFast: 53 days (range 33–79 days)
	Hospital stay, blood culture: 37 days (range 20–76 days)

IQR, interquartile range.

TABLE 11 Details of studies reporting changes in antimicrobial treatment plan

Author (year)	Reported changes in antimicrobial treatment plan
SeptiFast studies (non-comparative)	
Dierkes et al. (2009)[64]	SeptiFast: from pathogens identified by SeptiFast only, five (7.7%) patients had an adjustment of antimicrobial therapy
Lehmann et al. (2009)[66]	Blood culture: in 49 out of 467 (9.5%) episodes, antimicrobial treatment was changed
Maubon et al. (2010)[77]	SeptiFast: results would have significantly improved treatment in 11 (10%) patients, and prompted immediate antimicrobial therapy not given initially in three patients
Wallet et al. (2010)[81]	SeptiFast: on the basis of results, 8 out of 72 (11.1%) patients had an adjustment of antimicrobial therapy
Vrioni et al. (2011)[88]	SeptiFast: on the basis of results, 5 out of 33 (15.2%) patients had an adjustment of antimicrobial therapy
Grif et al. (2012)[90]	SeptiFast and concordant results from blood culture: 3 out of 33 (9.1%) patients had an adjustment of antimicrobial therapy
	SeptiFast and concordant results from samples from body sites: 5 out of 33 (15.2%) patients had an adjustment of antimicrobial therapy
Lodes et al. (2012)[92]	SeptiFast: on the basis of results, 25 out of 148 (16.9%) samples had an adjustment of antimicrobial therapy
Tschiedel et al. (2012)[96]	Patients with positive SeptiFast: 35 out of 75 (46%) had an adjustment of antimicrobial therapy
	Patients with negative SeptiFast: 5 out of 75 (6%) had an adjustment of antimicrobial therapy
Herne et al. (2013)[97]	SeptiFast: on the basis of results, 21 out of 54 (39%) positive cases had an adjustment of antimicrobial therapy
Mancini et al. (2014)[103]	Reports no between-group differences were observed in changes in management (propensity matching)
Markota et al. (2014)[104]	SeptiFast: on the basis of results, four (6.3%) samples had an adjustment of antimicrobial therapy
SeptiFast studies (comparative: RCTs)	
Rodrigues et al. (2013)[100]	SeptiFast: on the basis of results, 6 out of 17 (35%) patients had an adjustment of antimicrobial therapy
	Blood culture: on the basis of results, 7 out of 29 (21%) patients had an adjustment of antimicrobial therapy
	Between-group difference not reported
Idelevich et al. (2015)[110]	SeptiFast: on the basis of results, 7 out of 74 (9.5%) patients had an adjustment of antimicrobial therapy
	Blood culture: on the basis of results, 8 out of 76 (10.5%) patients had an adjustment of antimicrobial therapy
	Between-group difference not reported
Tafelski et al. (2015)[112]	SeptiFast and blood culture with MALDI-TOF MS: on the basis of results, 4 out of 41 (9.8%) patients had an adjustment of antimicrobial therapy
	Blood culture with MALDI-TOF MS: on the basis of results, 5 out of 37 (13.5%) patients had an adjustment of antimicrobial therapy
	Between-group difference not statistically significant: $p \geq 0.05$
IRIDICA studies	
Vincent et al. (2015)[116]	A panel of three independent experts (randomly selected from a pool of seven) would have recommended a change in management, including initiation of therapy, altered antimicrobial spectrum and/or change in duration of therapy, based on the IRIDICA results. The panel recommended a change in management in 41% of patients. Where IRIDICA tests were positive and blood culture results were negative, a change in management was recommended in 57% of patients; no values were provided for other scenarios

Mortality Seventeen of the SeptiFast studies,[64,66,74,78,80,85,89,90,92,94,100,103–105,110–112] one SepsiTest study,[114] one IRIDICA study[116] and one study evaluating both SeptiFast and SepsiTest[119] reported data on mortality. The majority of studies reported data for the whole patient cohort, as opposed to comparative data for the index and reference test. A summary of the mortality rates across all included studies is presented in *Table 12*. Across the RCTs, the SeptiFast RCT by Tafelski *et al.*[112] reported ICU mortality of 7 out of 41 (17%) participants in the SeptiFast group and 8 out of 37 (22%) participants in the blood culture group. The between-group difference was not statistically different ($p \geq 0.05$); the SeptiFast RCT by Idelevich *et al.*[110] reported that five (6.6%) participants in the blood culture group and three (4.1%) participants in the SeptiFast group died, but did not report when this occurred. The SeptiFast RCT by Rodrigues *et al.*[100] reported that the between-group difference in 28-day mortality was not statistically significant.

In summary, the current evidence for the impact any of the index tests evaluated against blood culture on intermediary and clinical outcomes, such as mortality and reduced length of stay in critical care units, is limited. A small number of RCTs of low methodological quality have not shown SeptiFast to produce a statistically significant improvement compared with blood culture and there are presently no RCTs comparing the other index tests (SepsiTest or IRIDICA) of interest with blood culture, or any RCTs comparing any of the index tests of interest in a head-to-head manner.

Additional information on matrix-absorbed laser desorption time-of-flight mass spectrometry

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:[30,122] one focusing on the time taken to identify microbial organisms from positive blood cultures[122] and one reviewing the performance of Sepsityper kit in conjunction with MALDI-TOF MS.[30]

Dixon *et al.*[122] identified 10 studies which provided evidence that MALDI-TOF MS is associated with faster identification of pathogens, usually 24 hours sooner 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'.

Morgenthaler and Kostrzewa[30] summarise data from 21 reports to assess the reliability of the Sepsityper kit in the rapid identification of bloodstream infection. It was reported that 'no relevant misidentification on the genus level was reported at a log (score) cut-off of 1.6', whereas 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.

TABLE 12 Details of studies reporting data on mortality

Author (year)	Reported mortality details
Single index test studies: SeptiFast (non-comparative)	
Dierkes *et al.* (2009)[64]	In-hospital mortality: 33%
Lehmann *et al.* (2009)[66]	30-day mortality: 33.8% of 467 episodes
Bloos *et al.* (2010)[74]	29.9% – location/time period NR
Regueiro *et al.* (2010)[78]	37.5% – location/time period NR[a]

TABLE 12 Details of studies reporting data on mortality (*continued*)

Author (year)	Reported mortality details
Tsalik *et al.* (2010)[80]	2.6% – location/time period NR
Josefson *et al.* (2011)[85]	30-day mortality: 4%
Alvarez *et al.* (2012)[89]	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)[90]	24-hour mortality: 61%
Lodes *et al.* (2012)[92]	43.2% – location/time period NR
Pasqualini *et al.* (2012)[94]	In-hospital: 12%
Mancini *et al.* (2014)[103]	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 be associated with lower mortality rates [3.13% (n = 2 deaths) in the prospective cohort and 14.71% (n = 10 deaths) in the retrospective cohort (p = 0.04)]
Markota *et al.* (2014)[104]	In-hospital mortality: 52.6%
Ozkaya-Parlakay *et al.* (2014)[105]	25.3% – location/time period NR
Idelevich *et al.* (2015)[110]	SeptiFast: 4.1% – location/time period NR
	Blood culture: 6.6% – location/time period NR
	Between-group difference: p = 0.719
Single index test studies: SeptiFast (comparative: RCTs)	
Rodrigues *et al.* (2013)[100]	28-day mortality SeptiFast: 53%
	28-day mortality blood culture: 59%
	Between-group difference: p = 0.765
Tafelski *et al.* (2015)[112]	ICU mortality, SeptiFast and blood culture with MALDI-TOF MS: 17%
	ICU mortality, blood culture with MALDI-TOF MS: 22%
	Between-group difference: not statistically significant (p ≥ 0.05)
Warhurst *et al.* (2015)[111]	28-day mortality: 14%
Single index test studies: SepsiTest	
Loonen *et al.* (2014)[114]	3.2% – location/time period NR
Single index test studies: IRIDICA	
Vincent *et al.* (2015)[116]	29% – location/time period NR
Two index test studies: SeptiFast and SepsiTest	
Schreiber and Nierhaus (2013)[119]	ICU mortality: 16%
	28-day mortality: 24%

NR, not reported; NS, not significant.
a Reporting discrepancy in article, text says 32.8% whereas tables say 37.5%.

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.[123] report the implementation of an evidence-based intervention that integrated MALDI-TOF MS, rapid antimicrobial susceptibility testing and near-real-time antimicrobial stewardship practices. A comparison of the results before and after testing was made. The mean hospital length of stay for survivors ($n = 100$) after bloodstream infection onset was 9.9 days in the pre-intervention group, compared with 8.1 days in the intervention group ($n = 101$; $p = 0.01$). 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 to 7.33; $p = 0.02$) and the intervention was associated with a hazard ratio for discharge of 1.38 (95% CI 1.01 to 1.88; $p = 0.04$). Total hospitalisation costs were US$45,709 in the pre-intervention cohort and US$26,162 in the intervention cohort.

A further paper[124] reported a pre–post quasi-experimental study that analysed the impact of MALDI-TOF MS with an antimicrobial stewardship team. The intervention ($n = 256$) decreased time to organism identification compared with previous treatment prior to MALDI-TOF with an antimicrobial stewardship team ($n = 245$) (55.9 vs. 84.0 hours; $p < 0.001$) and improved time to effective antibiotic therapy (20.4 vs. 30.1 hours; $p = 0.021$), optimal antibiotic therapy (47.3 vs. 90.3 hours; $p < 0.001$) and length of ICU stay (8.3 vs. 14.9 days; $p = 0.014$). The 30-day all-cause mortality was lower in the intervention arm than in the pre-intervention arm (12.7% vs. 20.3%; $p = 0.021$), as was length of hospitalisation (14.2 vs. 11.4 days; $p = 0.066$).

A study in Texas (USA) compared the outcomes of antibiotic-resistant Gram-negative bacteraemia in 112 patients admitted during January 2009 to November 2011 and 157 patients admitted during February 2012 to June 2013 following the introduction of an intervention (MALDI-TOF MS and antimicrobial stewardship).[125] Time to initiation of active treatment was 90 hours in the first group and 32 hours in the second group ($p < 0.001$). There were 33 (21%) deaths from any cause in the pre-intervention cohort and 10 (9%) in the intervention cohort. In multivariate logistic regression, the intervention was a significant predictor of survival (odds ratio 0.28, 95% CI 0.12 to 0.71; $p = 0.008$). A significant reduction in average total hospital costs was observed, from US$78,991 to US$52,693.

A quasi-experimental study[126] was conducted to evaluate the effect of introducing MALDI-TOF MS plus antimicrobial stewardship team review on the treatment of hospitalised patients in whom blood samples tested positive for coagulase-negative staphylococci ($n = 324$). Before the introduction of the intervention, 117 positive cultures (72%) were deemed to be contaminated and 46 (28%) were from patients with bacteraemia. The corresponding figures after the introduction of the intervention were 129 (80%) and 32 (20%), respectively. Following the introduction of MALDI-TOF MS plus antimicrobial stewardship team review, patients with bacteraemia received optimal therapy sooner (34.4 vs. 58.7 hours; $p = 0.032$) and exhibited a lower mortality rate than patients treated before the introduction of the intervention (3.1% vs. 21.7%; $p = 0.023$). In addition, following introduction of the intervention, patients whose blood samples were contaminated experienced a shorter duration of unnecessary antibiotic therapy (1.31 vs. 3.89 days; $p = 0.032$) and underwent fewer vancomycin trough assays (0.88 vs. 1.95; $p < 0.001$). However, rates of mortality, duration of hospitalisation stay, recurrent bloodstream infections and 30-day hospital readmissions were not significantly different.

A paper by Martiny and Debaugnies[127] reports that the use of MALDI-TOF MS resulted in the modification of treatment in 21 out of 157 adults and 1 out of 40 children.

The potential benefits associated with MALDI-TOF MS, albeit not from RCTs, is believed to be the motivation for the RAPIDO study,[31] which will report the required data for assessing the clinical effectiveness of MALDI-TOF MS, typically in conjunction with Sepsityper and blood culture compared with blood culture alone within clinical practice. It is envisaged that the results from this study will make the preceding data in this section largely redundant.

Chapter 3 Assessment of cost-effectiveness

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 of people who are suspected of having sepsis. As previously stated, earlier versions of the 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.

Methods

Electronic resources
A systematic search was undertaken 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 that may be present in the bloodstream in people who are suspected of having sepsis.

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

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

Sensitive keyword strategies using free text and, where available, thesaurus terms using Boolean operators and database-specific syntaxes were developed to search the electronic databases. Synonyms relating to the condition (e.g. sepsis) and the test (i.e. 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 *Chapter 2, Methods for reviewing effectiveness*, 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*.

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 cited the relevant articles. In addition, systematic keyword searches of the World Wide Web 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.

Studies were selected for inclusion according to predetermined inclusion and exclusion criteria. A summary of these criteria is 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.

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. Owing to these limitations the relevance of each study to the decision problem is discussed within *Descriptive summary of the study included in the review*, *Critique of the studies included in the review* and *Relevance of existing economic evaluations for National Institute for Health and Care Excellence decision-making*.

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 interventions listed below with 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 bloodstream infections in secondary care (i.e. departments and wards providing care for acutely unwell patients and/or critical care units) who required blood cultures	Those people not meeting the inclusion criteria
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	Other forms of economic evaluations

Results

Identified studies

A total of 89 citations were retrieved. Of these, 77 citations were identified via database searching 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.[89,103,128,129] These included an economic evaluation of the IRIDICA–PLEX-ID hybrid assay reported in a poster presentation (submitted by the company).[128] It was 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 *Chapter 1, IRIDICA BAC BSI*).
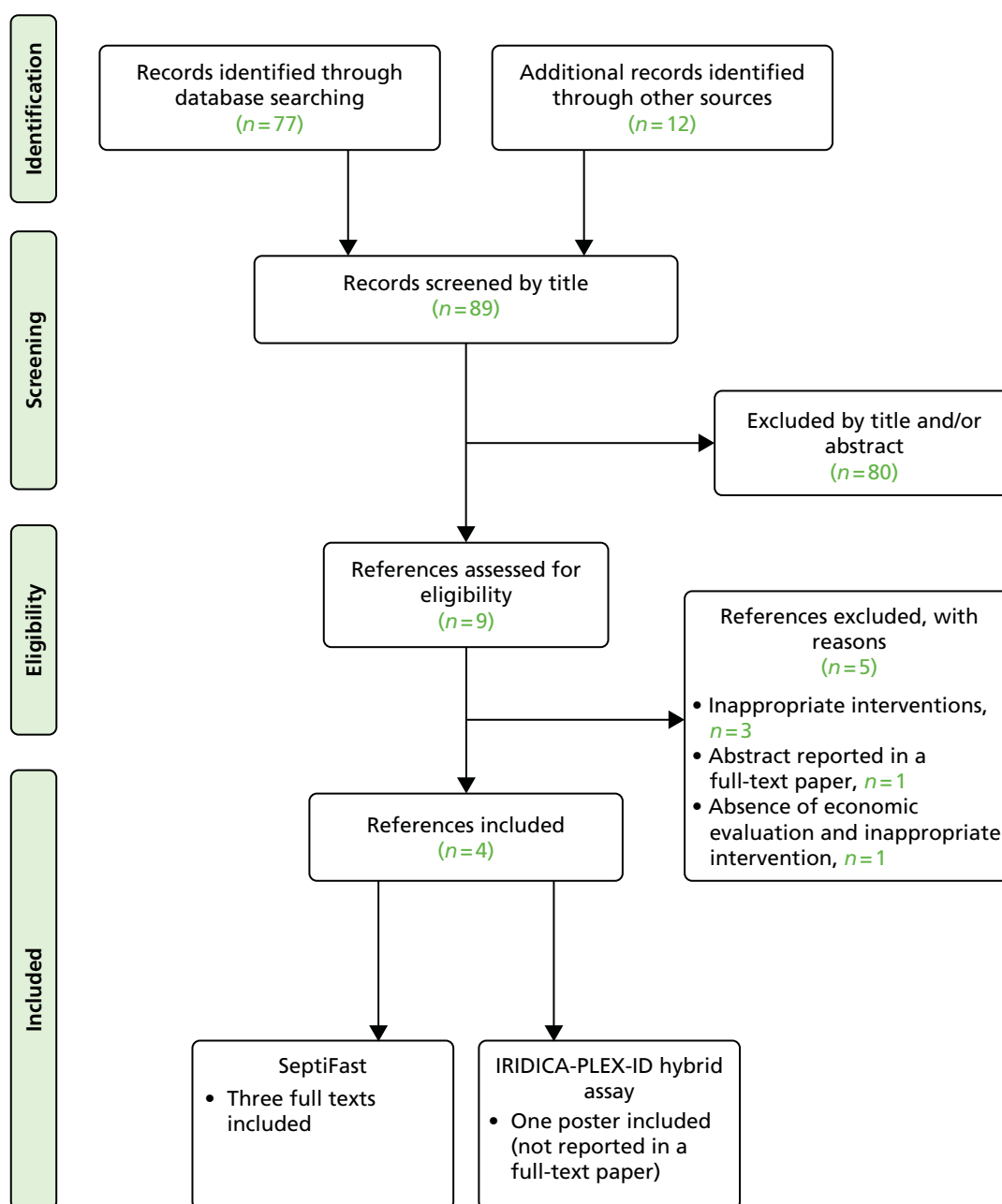
FIGURE 10 Study flow chart (adapted from Moher *et al.*[58]): economic review.

Five papers were excluded after retrieval of the full papers,[130–134] the rationale being that the results were published in full elsewhere,[130] they were other interventions[131–133] and there was an absence of an economic evaluation and inappropriate intervention.[134]

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[89,103,129] and one poster presentation[128]), three economic evaluations (full text) compared the addition of SeptiFast to blood culture with blood culture alone[89,103,129] and one compared the addition of the IRIDICA–PLEX-ID hybrid assay to blood culture with blood culture alone (poster presentation).[128] No economic evaluations of SepsiTest were identified. None of the four published economic evaluations was conducted in a UK setting. However, the RADICAL study,[116] used for the impact of the IRIDICA–PLEX-ID hybrid assay on treatment modification, included two UK (out of nine) sites.[116]

Two[103,129] out of the three SeptiFast studies were funded by Roche Diagnostics; it was unclear whether or not the third study[89] 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.*[103] included haematological patients with signs of systemic inflammatory response syndrome with suspected sepsis (SIRS-SS). Alvarez *et al.*[89] included patients diagnosed with severe sepsis and septic shock. Lehmann *et al.*[129] included all post-surgical and ICU patients with a sepsis episode (predominantly hospital-acquired infection), while Bilkovski *et al.*[128] included critically ill patients with suspected bloodstream infection.

Three studies were cost-minimisation studies.[89,103,128] Two were conducted within studies: a non-matched retrospective study evaluating SeptiFast[89] and a propensity score-matched study evaluating SeptiFast against blood culture.[103] The Alvarez *et al.* study[89] justified the use of cost minimisation given the absence of mortality data associated with the use of SeptiFast. The third cost minimisation study was undertaken using a decision tree model[128] and evaluated the IRIDICA–PLEX-ID hybrid assay by combining evidence from the RADICAL study[116] on the impact of the test in terms of treatment decision, and evidence from MALDI-TOF MS studies[123,124] 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 testing positive using the IRIDICA–PLEX-ID hybrid assay experience a reduction in length of stay.

Only one study was a cost-effectiveness (cost–utility) analysis and estimated the 'cost per incremental survivor' and the cost per quality-adjusted life-year (QALY) gained of introducing SeptiFast.[129] An algebraic model was constructed, which independently estimated the potential cost impacts and clinical outcomes associated with a change in treatment plan as a result of the earlier identification of inadequate treatment through the use of SeptiFast. Only positive SeptiFast results were considered to provide sufficient evidence to allow a treatment change, with the authors concluding that 'withdrawal of antimicrobial treatment upon a PCR negative result is not recommended'.[129] Cost savings and increased health associated with quicker adequate treatment were estimated assuming a relationship between a reduction of 1 day in inadequate treatment and changes in both length of stay and 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 USA.[66] In addition, the modelling uses pooled data on the impact of inadequate treatment on outcomes from two previously published studies[135,136] conducted in the USA.

TABLE 14 Key characteristics of economic evaluations included in the review

Parameter	Alvarez et al. (2012)[89]	Bilkovski et al. (2014)[128]	Lehmann et al. (2010)[129]	Mancini et al. (2014)[103]
Country	Spain	Unclear	Unclear	Italy
Study type	Within-study (observational retrospective non-matched study) economic evaluation	Mathematical model [based on the RADICAL (Rapid Diagnosis of the Critically Ill)[119] study and data from two MALDI-TOF MS studies]	Mathematical model (evidence from different sources combined)	Within-study (observational propensity score-matched study) economic evaluation
Economic evaluation	Cost minimisation	Cost minimisation	Cost-effectiveness/cost–utility analysis Cost per incremental survivor Cost per QALY gained	Cost minimisation
Rationale for the approach used	No difference in observed mortality	Not provided	NA	Not provided
Intervention	SeptiFast	IRIDICA–PLEX-ID hybrid	SeptiFast	SeptiFast
Comparator	BC	BC	BC	BC
Funder of the study	Unclear	Abbott Diagnostics	Roche Diagnostics	Roche Diagnostics
Target population and condition	Patients diagnosed with severe sepsis and septic shock	Critically ill patients with suspected BSI	Patients with a sepsis episode. Predominantly hospital-acquired infection	Haematological patients with signs of systemic inflammatory response syndrome with suspected sepsis
Setting	ICU	Majority ICU	Post-surgical and ICU patients	Haematology and bone marrow transplant units
Age	≈ 65 ± 14 years	60.4 ± 18.8 years	> 60 years	≈ 50 ± 14 years
Source used for the impact of the test on treatment modification	NA – within-study economic evaluation	RADICAL study[116]	Evidence collected prospectively from five hospitals (two German, one Italian, one Spanish and one US hospital)[66]	NA – within-study economic evaluation
Source used for the impact of the test on clinical outcomes (mortality, length of stay)	NA – within-study economic evaluation	Two studies of MALDI-TOF MS, in addition to an antimicrobial stewardship programme[124,125]	Pooled data on the impact of inadequate treatment on outcomes from two previously published studies[135,136] conducted in the USA	NA – within-study economic evaluation

TABLE 14 Key characteristics of economic evaluations included in the review (*continued*)

Parameter	Alvarez et al. (2012)[89]	Bilkovski et al. (2014)[128]	Lehmann et al. (2010)[129]	Mancini et al. (2014)[103]
Study perspective	Health-care perspective	Health-care perspective	Health-care perspective	Health-care perspective
Discounting	NA	NA	Not stated	NA
Time horizon	NA	NA	Lifetime	NA
Cost categories included in the economic evaluation	Antibiotic treatment ICU stay Ward stay SeptiFast test	ICU stay Ward stay IRIDICA–PLEX-ID hybrid	SeptiFast test	Diagnostic and laboratory 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
Cost of the intervention	€183 (based on seven patients) per patient including reagent cost, personnel cost and imputable structural costs	US$250	€300	€178.75 per sample (average of four samples plus positive and negative controls per each run, including both reagents and personnel costs)
Unit costs for other resource use	Stay in ward: €273 Stay in ICU: €1058 Cost of antibiotics taken directly from hospital – values not reported	Non-ICU stay per day: US$2122 ICU stay per day: US$3500	NA	Taken 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

Parameter	Alvarez et al. (2012)[89]	Bilkovski et al. (2014)[128]	Lehmann et al. (2010)[129]	Mancini et al. (2014)[103]
Results	Total costs (test vs. BC) per patient: €32,228 vs. €42,198; p = 0.05 (saving of €9970) 96.3% probability of cost savings	Total costs (test vs. BC): US$19,375,716 vs. US$20,499,088 per 422 tests	€11,44 (95% CI €9321 to €14,977) per incremental survivor €3107 (95% CI €2523 to €4055) per QALY gained	Total costs (test vs. BC) per patient: €1579.80 (median €1075.47) vs. €2010.53 (median €1105.18); p = 0.05 (saving of €430.73)
Breakdown of clinical results	ICU length of stay (control vs. test) (31.0 ± 19.4 vs. 22.9 ± 29.9 days) Hospital length of stay (21.3 ± 23.4 vs. 18.3 ± 21.4 days) ICU length of stay (survivors) (24.1 ± 21.9 vs. 18.3 ± 11.4 days) Number of antibiotics used per patient (5.1 ± 3.1 vs. 4.2 ± 2.2)	4.2 days saved in hospital stay in patients with a positive test (1.6 days in all patients) 1.8 days saved in ICU stay in patients with a positive test (0.7 in all patients)	80.5 days (95% CI 48 to 113 days) potential earlier treatment Absolute reduction in mortality of 2.6%	NA
Breakdown of cost results	Antibiotic treatment costs (test vs. control): €2812 vs. €3,576; p < 0.05 ICU costs: €24,246 vs. €32,798; p < 0.05 Ward costs: €5988 vs. €5824; p < 0.05	Hospital cost (per 422 tests) (test vs. control): US$19,185,816 vs. US$20,414,688		Classical diagnostic and instrumental procedures assays (test vs. BC): €652.79 vs. €625.66; p = 0.68 Medication costs: €927.01 vs. €1384; p = 0.02

BC, blood culture; BSI, bloodstream infection; NA, not applicable.

Although not clearly stated by the authors, it is believed by the authors of this report that Mancini *et al.*[103] and Alvarez *et al.*[89] report results from an Italian and Spanish setting, respectively. In the case of the studies by Lehmann *et al.*[129] and Bilkovski *et al.*,[128] the country on which the analysis was based is unclear, as the data came from multicentre studies.

The economic evaluation was conducted in patients in the ICU in three studies.[89,128,129] All studies appear to use a hospital (health-care payer) perspective, although this was not explicitly stated in two studies.[103,128] Uncertainty was examined in two studies.[89,129] Only one study used QALYs as a measure of benefit.[129]

The cost of performing SeptiFast varied between studies and ranged from €178.75[103] (£128.70 assuming an exchange rate of €1 to £0.72[137]) to €300[129] (£216). One study evaluated the IRIDICA–PLEX-ID hybrid assay and assumed a cost of US$250 per test[128] (£160.67 assuming an exchange rate of US$1 to £0.64).[137]

Two studies[89,103] 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[103] 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.[76,89,128] 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 being outweighed by the savings in antibiotics and/or hospital costs.[89,103,128] Mancini *et al.*[103] reported an overall saving of €430.73 per patient receiving a SeptiFast test and blood culture compared with those diagnosed using only blood culture (€1579.80 vs. €2010.53), considering savings in diagnostic and instrumental assays, and medications (including antibiotics, antimycotics, antiviral agents and other drugs). Alvarez *et al.*[89] reported a saving of €9970 per SeptiFast test, with the majority of savings achieved based on a reduction in ICU length of stay. Bilkovski *et al.*[128] reported a total saving of US$1,123,372 over 422 patients tested (equating to a saving of £2662 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.*[129] reported that the cost of the SeptiFast test could be recovered if the daily medical costs were above €717 and suggested that this was likely to be the case.[129] The authors reported the cost per incremental survivor and cost per QALY gained to be €11,477 (95% CI €9321 to €14,977) and €3107 (95% CI €2523 to €4055), respectively.

Critique of the studies included in the review

The Lehmann *et al.*[129] economic evaluation, which compared the use of SeptiFast and 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 that may restrict the generalisability to UK practice, not only because none of the hospitals contributing to the study was located in the UK. The data used in the Lehmann *et al.*[129] 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] Therefore, the extent to which the inadequate treatment observed in Lehmann *et al.*[66] is generalisable to current practice in the UK is not known. 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.*,[129] could be potentially confounded. Lehmann *et al.*[129] 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[138] 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.[138] 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, in the original Lehmann et al.[66] 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 days for the ICU or surgical ward group and 10.6 days for the remaining attendance method. It is unclear if the evaluation by site was preplanned or if 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.[89,103,110,112,139]

The cost-minimisation study conducted by Alvarez et al.[89] 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. Alvarez et al.[89] 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 days; $p < 0.05$), hospital length of stay (21.3 ± 23.4 vs. 18.3 ± 21.4 days; $p < 0.05$) and ICU length of stay (24.1 ± 21.9 vs. 18.3 ± 11.4 days; $p < 0.05$). 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 €9970 (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 because of the retrospective nature of this study, the small sample size of each arm (48 patients in the intervention group and 54 patients in the control group) and the imbalances between patient characteristics, as shown in Table 15. It is also unclear whether patients were randomly selected to receive SeptiFast or whether these patients were selected based on their characteristics. Although the authors reported no differences in terms of patients' ages, sex 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 that could impact on ICU length of stay (main source of savings). It should be noted that

TABLE 15 Comparison of baseline characteristics between the control and intervention group (adaptation of table 2 in Alvarez et al.[89])

	Group	
Baseline characteristics	Control ($n = 54$)	Intervention ($n = 48$)
Age (years), mean ±SD	65 ± 14.7	54 ± 12.9
Sex, % male	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

CNS, central nervous system.
Paper only reported the initial diagnosis for 47/48 people who received the intervention.

no difference in ICU or hospital length of stay was observed in other comparative studies (RCT or propensity-matched score studies).[103,110,112,139] The authors report a saving in antibiotics (excluding cost of the test) of €764 per patient [as a result of a reduction in the number of antibiotics used per patient of 0.9 (from 5.1 to 4.2)]. The authors do not say which antibiotics were used in the study, or for how long. It should be noted that, in England, antibiotics are typically given as a 5- to 7-day course, with a daily cost around £50. The failure rate of the SeptiFast test was not considered.

The second cost-minimisation study, by Mancini et al.[103] evaluated the use of SeptiFast with 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) towards a 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, it reduced the precision by decreasing the sample size and could also introduce bias, as the estimate is no longer the effect of treatment in the treated subjects, but the effect of treatment in those treated subjects for whom a control was found.[140] Using the tight calliper of 0.05, the authors found a mortality rate of 3.13% in the intervention group and 14.71% in 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 events were needed to demonstrate a 10.6% difference in mortality (9.7% in the intervention group vs. 20.3% in the control group), which is greater than the numbers analysed in Mancini et al.[103] The results from this study therefore need to be interpreted with caution. It should also be noted that the PCR test was implemented under optimal conditions. The authors found 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, the cost of antibiotics was higher in patients receiving SeptiFast (€11.78). In contrast, the costs of antimycotics (commonly called antifungals in England) (€376.62), antiviral agents (€1.65) and other drugs (€91.39) were reduced. Although the two cohorts were propensity matched, it is unclear whether or not the suspected organisms in the two cohorts were matched, which may affect the antibiotics prescribed. In the control arm, antimycotics represented approximately 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 were selected based on their characteristics.

Finally, the Bilkovski et al. study[128] is a decision tree using evidence from the RADICAL study[116] on the impact of the IRIDICA–PLEX-ID hybrid assay (referred to by the authors as the PCR/ESI-MS) in terms of treatment decision and studies on MALDI-TOF MS[123,124] for the impact of the test on clinical outcomes. This study was available only as a poster presentation and, therefore, it is difficult to judge its quality. However, the External Assessment Group notes some inconsistencies. In the RADICAL study, Vincent et al.[116] reported that, among the 529 patients included, the overall mortality rate was 29%, suggesting that about 375 patients survived hospital discharge. However, the economic evaluation conducted by Bilkovski et al.[128] is based on 422 patients and includes only 290 patients surviving to hospital discharge. Patients who tested positive using the IRIDICA–PLEX-ID hybrid were assumed to experience a reduction in hospital and ICU length of stay because of the earlier availability of results and potential therapy changes based on data from two previous studies of MALDI-TOF MS.[123,124] 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 there may be unintended consequences. Importantly, studies on MALDI-TOF MS[123,124] used in the economic evaluation evaluated the impact of MALDI-TOF MS in combination with an antimicrobial stewardship programme compared with blood culture prior to the programme. Hence, the impacts of MALDI-TOF MS in these studies are highly likely to be confounded by the simultaneous introduction of an antimicrobial stewardship programme. These studies were also non-randomised in nature and, therefore, subject to

potential biases. Bilkovski et al.[128] 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, respectively, in all patients and of 4.2 days and 1.8 days, respectively, in patients with positive IRIDICA-PLEX-ID results. It is unclear how the model estimate relates to the impact of the test in practice and whether or not 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.[89,100,103,110,112] Test failure was not considered in the analysis.

Relevance of existing economic evaluations for National Institute for Health and Care Excellence 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. However, 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 with 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 studies included economic evaluations.[89,103] However, there are limitations because of the retrospective nature of these studies and potential biases associated with patient selection. Notably, the study by Alvarez et al.[89] is believed to be highly confounded, with large imbalances between groups in terms of initial diagnosis: in the control group ($n = 54$), 20 patients had had heart surgery and four patients had polytrauma/head injuries whereas, in the SeptiFast group ($n = 48$), only two patients had had heart surgery but 20 suffered from polytrauma/head injuries.
- Two studies used modelling approaches to assess the impact of SeptiFast[129] or the IRIDICA–PLEX-ID hybrid assay,[128] compared with blood culture, on treatment modification and the resulting effect in terms of inadequate therapy or rapid identification. The results produced contradict the evidence of the impact of the tests on clinical outcomes. The evidence so far is inconclusive, despite five comparative studies comparing the impact of SeptiFast with that of blood culture, with or without MALDI-TOF MS, on mortality and hospital/ICU length of stay.[89,100,103,110,112] In the SeptiFast modelling study[129] the impact of the test on treatment change and 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] Bilkovski et al.[128] based their model of the IRIDICA-PLEX-ID hybrid assay on the results of two earlier MALDI-TOF MS studies.[123,124] Both of these studies[128,129] suffer from limitations: not only were they non-randomised, and therefore subject to biases, but they evaluated the use of MALDI-TOF MS in combination with an antimicrobial stewardship programme only in patients with positive blood cultures. Furthermore, the potential impact of the test on treatment modification was estimated retrospectively, which may have introduced a further source of bias.
- The cost of SeptiFast differed among the three studies[89,103,129] and does not necessarily reflect the most likely cost estimated by the External Assessment Group. Similarly, the cost of the IRIDICA–PLEX-ID hybrid assay[128] 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.,[103] 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.[89,100,103,110,112]

- Mancini *et al.*[103] 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 antifungals. It is unclear whether or not these reductions are generalisable to the NHS in England.
- The identified economic evaluations focused 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 by Leone *et al.*,[35] who evaluated the effects of de-escalation in patients with sepsis. Furthermore, any detrimental effects that may occur as a result of 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 conditions in the majority of identified economic evaluations[103,129] 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 acknowledges the difficulty of robustly quantifying such benefits.
- It is also unclear whether or not patients were preselected in the studies.
- Finally, the failure rates within interventions were not considered. A recent UK study conducted by Warhurst *et al.*[10] suggests a test failure rate of 7% for SeptiFast. An alternative study reported failure rates of 22.9%.[99]

Independent economic assessment: conceptual model and methods

The conceptual model developed by the External Assessment Group was relatively simplistic because of 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[141] 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 because of 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 patients, 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 patients, 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.

Although the costs of the tests and comparators can be estimated relatively well from current data, there are no conclusive data on any other parameter listed in the bullet points above that were identified in 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: 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 because of 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 because of the potential fatal consequences if treatment was withdrawn from a patient with sepsis. Acknowledged reasons for a false-negative result include the test being unable to detect the pathogen or the quantity of the pathogen being below the test's limit of detection. Similarly, tests that 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.
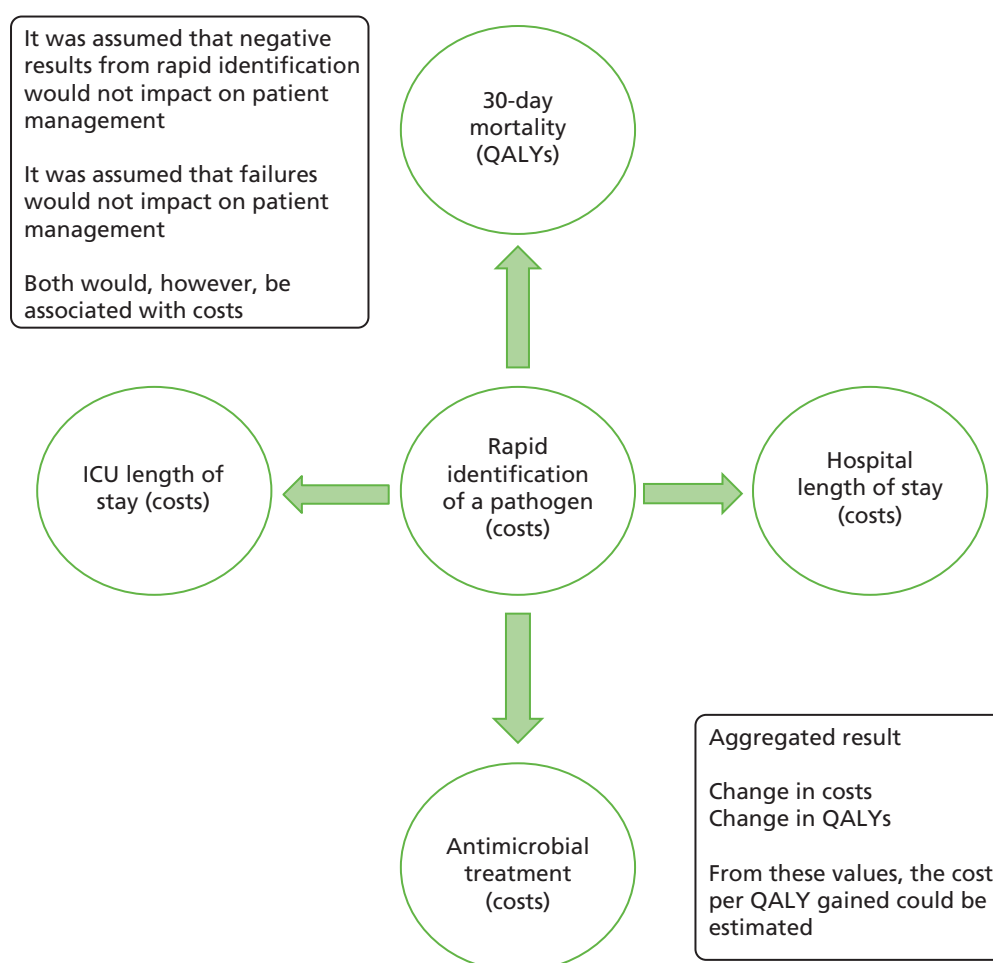
FIGURE 11 The components within the conceptual model.

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 in which 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 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 value than in estimating a value in the absence of data (as was requested in base case 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 no additional QALY gain was associated with a reduction in ICU duration of stay. The results are presented allowing for a mixture of both net mortalities and of net reduced ICU stay. In an alternative 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 with MALDI-TOF MS based on published literature.
5. Analyses of data taken from studies in which more than one intervention were compared directly.

Given the large divergence in results produced by base cases 1 and 2, the External Assessment Group decided that probabilistic sensitivity analyses would provide spurious accuracy 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 among subgroups resulted in the External Assessment Group providing only an overall measurement of cost-effectiveness rather than by subgroup. Although the cost-effectiveness may differ among subgroups – for example, a neonate would be expected to accrue more QALYs than an adult – these do not affect the fundamental uncertainty of whether or not the interventions would be associated with any key patient outcome.

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, that each positive test was associated with a 0.1-day reduction in ICU length of stay, a 0.3-day 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:

$$£400 + (\{(-0.1 \times £1057) + [(-0.3 - -0.1) \times £275] + -£50\} \times 20\%) = £357.86. \qquad (11)$$

Note that these values are for illustrative purposes and are not necessarily those used in the modelling exercises that are detailed in later sections.

The incremental QALYs have been calculated assuming 11.32 discounted QALYs per 30-day mortality avoided (see *The quality-adjusted life-year gains associated with preventing a 30-day mortality*). 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 reduction following a positive test was 5% and the rate of positivity was 20%, then the estimated number of deaths prevented would be 13% × 5% × 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 *Model parameters assumed for base case 1*, separate values for these were provided by the clinical experts.

Independent economic assessment: populating the model

General model parameters

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

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, large, high-quality study set in England. In this study there was a central hub, in Salford, with a SeptiFast machine that 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 because of 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 in the remaining sites.

The sites' requirements sum to 36.48 tests per month, although results were provided for only 922 out of 1006 samples: the SeptiFast had a failure rate of 69 out of 1006 (7%) and a further 15 samples were lost for clinical reasons. The Warhurst *et al.* study[10] included only health-care-acquired sepsis. Based on clinical opinion, it was assumed that 50% of sepsis cases are health care acquired and 50% are community acquired, and thus, to include both types of sepsis, the number of tests required was doubled to 72.96 each month, or 2.40 tests each day. It was assumed that two tests would be analysed on 60% of days and three tests would be analysed on 40% of days. However, on clinical advice, it was further assumed that testing would be carried out on only 5 days a week; therefore, the numbers of tests were divided so that there were three

TABLE 16 Calculation of the estimate of the daily number of tests undertaken

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

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 on blood that was sampled on a Friday evening but which 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.

Assuming that the number of samples that need to be analysed each day is 17

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 1 day, assuming three runs per day, although it is noted that SeptiFast could process 21 tests over three 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 7 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.

Assuming that the number of samples that need to be analysed each day is 68

In this example, the value was set to approximately 28 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 7 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.

The estimated costs of the interventions and matrix-absorbed laser desorption/ionisation time-of-flight mass spectrometry

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 7-year period of use. Annual costs associated with the maintenance of the machines were incorporated.

For each intervention, the cost will be dependent on whether or not 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 generic PCR machines. 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. In both cases consumable costs would be incurred.

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, value-added tax (VAT) is not included in economic evaluations.[141] 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 the costs of the test are lower than those assumed in the analyses, then the incremental cost-effectiveness ratio (ICER) would be lower than those presented with all other variables held constant.

The costs associated with SeptiFast

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

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

The key consumables required to run a test, along with their list price, are shown in *Table 18*.

Roche Diagnostics estimated the cost per test based on the number of tests per run, assuming one daily run and a 5-day working week. Running more tests per run reduces the average reagent cost per sample because of 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 19* it can be calculated that the marginal cost of one additional sample in a run is £122.00.

TABLE 17 The instruments required to run SeptiFast and their list prices

Instrument	Category number	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	3060.00
MagNA Lyser Instrument (230 V)	03 358 976 001	4500.00
Multi-Colour Compensation Set	4484355001	536.76
SeptiFast Cooling Block	4555864001	300.45

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

Product name	Category number	List price (£)	Number per item
LightCycler M Grade Capillaries	3612066001	883.93	768
LightCycler SeptiFast Kit, CE	4469046001	1422.62	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

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 (2.39, 17 and 68) and for the two purchasing scenarios (where the SeptiFast platform does or does not need to be purchased). In both purchasing scenarios, consumable costs will be incurred.

The costs associated with SepsiTest

As previously detailed (see *Chapter 1, SepsiTest*), 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. Owing 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 reports 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 of 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 in one run. The costs of the controls were not provided by Molzym Molecular Diagnostics.; these were estimated to be £104.17 for the pair, which is the cost of two reactions. This is likely to underestimate the cost as additional costs associated with spiking blood to produce the positive control have not been included, although this omission is unlikely to affect the conclusions of the report.

The cost of additional sequencing following a positive SepsiTest was assumed to be €11 for bacteria and €5.50 for fungi based on information supplied by Molzym Molecular Diagnostics. These costs include VAT at 19% and are equivalent to €9.24 and €4.62, respectively, once VAT is deducted. Assuming an exchange rate of €1 to £0.72,[137] these values were calculated as £6.66 and £3.33, respectively.

The costs of the machinery required to undertake PCR testing and Sanger sequencing were estimated from data provided by Molzym Molecular Diagnostics. These costs are reproduced in *Table 21*. The data were assessed by a clinical expert advising the External Assessment Group and were deemed appropriate. Therefore, it was considered not unreasonable to assume a cost of €60,000 (a high estimate based on the values in *Table 21*) to purchase the equipment needed to undertake PCR and to cover any maintenance required. This is equivalent to £43,200, assuming an exchange rate of €1 to £0.72.[137]

In order that the profile of bacteria and fungi was 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 (see *Figure 6*), it was assumed that SepsiTest had a sensitivity of 0.48 and a specificity of 0.86, which, when combined with an 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 is not dependent on throughput.

TABLE 20 Estimated average costs per SeptiFast test

Scenario	Average cost per test assuming samples per day calculated from Warhurst *et al.*[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[a]	205.54	160.52	154.28
Assuming no additional machinery is needed	201.23	159.91	153.67

a Assuming that the intervention costs were spread over a 7-year period.

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

	Price (€)
Apparatus	
Thermomixer (24 × 2.0-ml tubes, adjust at 37 °C, 56 °C and 70 °C)	≈2000
Cooling racks for 1.5-ml tubes (adjust at 4 °C and –20 °C)	≈50
Vortexer (e.g. VWR, Germany)	≈170
Bench-top microcentrifuge (≥ 13.000 rpm, ≥ 12.000 × g)	≈1100
Clear work places	
• UV workstation or	≈3000
• UV laminar flow [e.g. BDK UVF, Germany (optional)]	≈8000
One 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	
• PCR cycler with	≈3000
• Gels analysis system or	≈4000
• Real-time PCR cycler (ramp 2 °C per second)	≈15,000
Sequencing analysis as service (GATC, Germany)	≈5 per sequencing
ABI 310 (Applied Biosystems, CA, USA) refurbished	≈30,000
Plastic ware	
Pipette tips with aerosol filter (e.g. Biosphere®, Sarstedt AG & Co., Germany)	
PCR tubes	
Chemicals	
DNA decontamination: DNA Exitus®, Applichem, Germany	
Agarose gel (2%) for standard PCR	
A container for plastic waste (pipette tips, vials, tubes) and another for liquid waste, autoclavable	
Others	
Sterile disposables	
Gloves (e.g. Kimberly-Clark, Germany)	
Sleeves (e.g. Cardinal Health, Ireland)	
Bouffant covers (e.g. VWR, Germany)	
Overshoes (e.g. hygi, Germany)	

rpm, revolutions per minute; UV ultraviolet.

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

The costs associated with an IRIDICA test
Abbott Diagnostics reports 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 the control test needs to be changed every 24 hours. These costs are summarised in *Table 23*. According to Abbott Diagnostics, it is possible to analyse 23 samples per day, assuming that one control is used at the start of the day along with five samples and that subsequent runs do not need a control and therefore six samples can be analysed simultaneously. The External Assessment Group comments that, although it is technically possible to conduct four runs, each taking slightly < 6 hours, in a 24-hour period, this is unlikely to be possible in practice. Therefore, the External Assessment Group assumed that 17 tests per day represents the limit of an IRIDICA machine. The estimated average costsper IRIDICA test are provided in *Table 24*.

The costs associated with a matrix-absorbed laser desorption time-of-flight mass spectrometry system
The costs associated with MALDI-TOF MS were provided by Bruker UK Limited (Coventry, UK) (Erika Tranfield, Bruker UK, 2015, personal communication). It was assumed that these costs would be generalisable to other MALDI-TOF MS systems such as bioMérieux's VITEK® MS system (Marcy-l'Étoile, France). 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 lysis 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, 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 are 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 3–5 maintenances per year is £17,000. It was assumed that the higher number of preventative maintenances applies.

It is noted that MALDI-TOF MS can be used for many investigations other 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.

TABLE 22 Estimated average costs per SepsiTest

Scenario	Average cost per test assuming samples per day calculated from Warhurst *et al.*[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[a]	149.53	112.29	108.55
Assuming no additional machinery is needed	142.48	111.36	108.30

a Assuming that the intervention costs were spread over a 7-year period.

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

TABLE 24 Estimated average costs per IRIDICA test

Scenario	Average cost per test assuming samples per day calculated from Warhurst et al.[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[a]	314.61	203.52	203.52
Assuming no additional machinery is needed	270.89	197.35	197.35

a Assuming that the intervention costs were spread over a 7-year period.

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 these samples would undergo 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 *Assuming the numbers of samples that need analysing based on the number observed in a recent clinical study*), which is 0.21 tests per day. When the assumed number of blood samples that need to be analysed was increased 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. The estimated costs of MALDI-TOF MS are provided in *Table 25*.

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

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.

The assumed failure rates of the interventions
Both SeptiFast and IRIDICA use internal controls that 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.*[99] Data on the failure rate of IRIDICA have been reported in Metzgar *et al*,[117] and indicate a rate of (confidential information has been removed). 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 naive pooling of failure results from Warhurst *et al.*[10] (69 failures) and from Paolucci *et al.*[99] (100 failures) divided by the numbers of samples analysed in all of the SeptiFast versus blood culture trials combined (11,659 samples), 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 (confidential information has been removed) was assumed based on a naive pooling of data from all of the studies assuming no failures in any study but Metzgar *et al.*[117]

Although Warhurst *et al.*,[10] Paolucci *et al.*[99] and Metzgar *et al.*[117] explicitly stated that any failures were excluded from analyses of diagnostic accuracy, it is not clear if 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.

TABLE 25 Estimated average costs per MALDI-TOF MS

Scenario	Average cost per test assuming samples per day calculated from Warhurst *et al.*[10] (£)	Average cost per test assuming 17 samples per day (£)	Average cost per test assuming 68 samples per day (£)
Assuming a MALDI-TOF MS machine needs to be purchased[a]	232.39	35.35	11.09
Assuming no additional machinery is needed	114.88	18.78	6.94

a Assuming that the intervention costs were spread over a 7-year period.

No data were identified on the failure rate of SepsiTest. Rather than assign an arbitrary value to this parameter, the value was set to zero with the acknowledgement that this was likely to be favourable to SepsiTest.

The quality-adjusted life-year gains associated with preventing 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 that the discounted QALY gains for neonates and children would be greater than those for adults because of their longer life expectancy, although the exact increase is uncertain.

Although there is evidence that the survival after a sepsis episode may be lower than in the general population,[142–144] for simplicity we assumed that survival of patients with sepsis was comparable with that of the general population. National life tables for England and Wales for the period 2011–13[145] were used to estimate the life expectancy of a typical patient, assuming an age of 58 years and a sex split of 60%/40% (male/female) as reported in Warhurst *et al.*[10]

Patients were assumed to have a utility value of 0.68 based on the European Quality of Life-5 Dimensions score reported by Cuthbertson *et al.*[142] at 5 years after a severe sepsis episode, which is similar to the value reported by Drabinski *et al.*[146] If the utility predicted for the general population for an age and sex profile[147] 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.[141]

The assumed cost of 1 day's treatment in an intensive care unit

This value was calculated from NHS reference costs.[148] 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, 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 for service code CCU002 [surgical adult patients (unspecified specialty)] selected, which was £987 per day.

The assumed cost of 1 day's treatment in a standard hospital ward

This value was calculated from NHS reference costs,[148] assuming that the average excess bed-day cost per non-elective impatient of £275 was appropriate.

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 18 g of piperacillin/tazobactam per day or 3 g of meropenem per day was an appropriate empirical treatment for typical sepsis patients. Using *British National Formulary* costs,[149] these prices were estimated to be £51.60 per day (assuming 4.5 g every 6 hours) for piperacillin/tazobactam and £48.00 (assuming 1 g 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.

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

It was assumed that the 28-day mortality rate reported in a recent health technology assessment,[111] 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 alternative 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] who reported hospital mortality rates of 29% when 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.

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 (see *Chapter 2, Mortality*), length of stay in ICU or length of stay in hospital (see *Chapter 2, Duration of intensive care unit and/or hospital stay*). One study of a propensity score-matched design was identified[103] and indicated a significant reduction in the costs of empirical therapy, but this study had a number of limitations. First, the study population was of haematological patients who were prescribed empirical antifungal drugs, which is not typical for a suspected sepsis patient. Second, 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 400 mg.[149]

Data were found showing that the time to therapy modification was much shorter following the introduction of SeptiFast (18.8 hours compared with 38.3 hours),[112] 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*. Therefore, it was assumed that the costs of antimicrobials were unchanged in the base case.

Thus, in base case 1, it was assumed that the results did not differ depending on whether an intervention was used or was not used. An analysis was undertaken to estimate the reduction in antimicrobial costs needed in order for an intervention to be cost neutral.

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 if the benefits anticipated were realised. 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 assumed the same values for SeptiFast, IRIDICA and SepsiTest. The values for MALDI-TOF MS were typically less favourable than for the three interventions as information on the pathogen would be provided at a later time point than for the interventions. The average values from the clinical experts shown in *Table 26* relate to the situation that an intervention is positive and the subsequent blood culture is positive; those in *Table 27* relate to the situation that an intervention is positive and the subsequent blood culture is negative. Note that monetary savings in antimicrobial costs were transformed into a percentage reduction, assuming a typical course of treatment cost of £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, in which blood culture is assumed to be the gold standard, and casts uncertainty over meta-analyses in which this assumption is made.

TABLE 26 The parameter values assumed in base case 2 when the intervention was positive and the subsequent blood culture was positive

Parameter	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

Parameter	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%

Consideration was given to attempting to construct a distribution to represent the values provided by the clinicians. However, we decided against this as it was not clear that it would provide significantly better data than an aggregate value. In addition, it would necessitate analysis at the individual clinician level as well as further assumption regarding the distribution type and the range surrounding the mid-point answers provided.

Data from individual clinicians were used in sensitivity analyses. These values are shown in *Tables 28–31* for the three interventions and MALDI-TOF MS when the blood cultures were subsequently positive, and in *Tables 32–34* for the three tests when the subsequent blood cultures were negative.

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

Clinician	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
1	−0.2	−0.5	−5%	−3%
2	0	−1	−15%	−1%
3	−0.1	−0.2	−£40 (−11%)	−0.1%
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%)
5	−1	−3	−£175 (−50%)	−2%
6	−0.001	0	−18%	0%
7	−0.45	−1.15	−25%	−15%

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

Clinician	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 antimicrobial drugs	Net effect on 30-day mortality
1	−0.2	−0.5	−5%	−3%
2	0	−1	−15%	−1%
3	−0.1	−0.2	−£40 (−11%)	−0.1%
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%)
5	−1	−3	−£175 (−50%)	−2%
6	0	0	0%	0%
7	−0.9	−2.3	−70%	−20%

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

Clinician	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
1	−0.2	−0.5	−5%	−3%
2	0	−1	−15%	−1%
3	−0.1	−0.2	−£40 (−11%)	−0.1%
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)
5	−1	−3	−£175 (−50%)	−2%
6	−0.001	0	−20%	0%
7	−1.35	−3.1	−80%	−25%

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

Clinician	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
1	−0.1	−0.2	−2%	−1%
2	0	−1	−15%	−1%
3	No answers provided			
4	0	0	0	0
5	−0.5	−1.5	−£100 (−29%)	−1%
6	0	0	−10%	0
7	−0.45	−1.85	−30%	−15%

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

Clinician	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
1	–1	–1	–15%	–8%
2	0	–1	–15%	–1%
3	0	–1.5	–£80 (–23%)	0%
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%)
5	0	–1	–£700 (–100%[a])	–0%
6	–0.05	0.0	–25%	0%
7	–0.45	–1.15	–25%	–15%

a The percentage reduction was capped at 100%.

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

Clinician	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
1	–1	–1	–15%	–8%
2	0	–1	–15%	–1%
3	0	–1.5	–£80 (–23%)	0%
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)
5	0	–1	–£700 (–100%[a])	–0%
6	0	0	0%	0%
7	–0.9	–2.3	–70%	–20%

a The percentage reduction was capped at 100%.

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

Clinician	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
1	–1	–1	–15%	–8%
2	0	–1	–15%	–1%
3	0	–1.5	–£80 (–23%)	0%
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)
5	0	–1	–£700 (–100%[a])	–0%
6	0.05	0	–27%	0%
7	–1.35	–3.1	–80%	–25%

a The percentage reduction was capped at 100%.

Independent economic model: results

As previously stated, the results will be presented in three broad categories: (1) base case 1, in which only data from the published literature considered appropriate are included in an economic evaluation to estimate the cost-effectiveness of each test; (2) base case 2, in which parameters were populated from estimates by clinical experts in order to estimate the cost-effectiveness of each test; and (3) 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 or £30,000 per QALY). In addition, supplementary analyses 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 is presented. It is acknowledged that this is likely to underestimate the gains in mortality prevented associated with neonates, but it was deemed that this would not affect the conclusion, which would remain uncertain.

Results from base case 1

The estimated costs and estimated QALYs when the machinery required for the intervention needs to be purchased are shown in *Table 35* and when no additional machinery needs to be purchased are provided in *Table 36*. For brevity, these results are presented only 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 owing to the assumed lack of QALY gain. The 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 needs to be purchased

Test	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

Test	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 estimate the reduction in antimicrobial costs needed per test in order that the introduction of the intervention would 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.

If it is assumed that a reduction in antibiotic costs will be achieved only in the event of a positive test, then, assuming the positivity rates calculated for each test based on their estimated sensitivity and specificity values, the costs of the tests cannot be recouped from reduced antimicrobial treatment costs alone.

Results from base case 2

Results are presented separately using the average value from all clinician responses and by individual clinician. The results are also differentiated 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 those assuming a mortality rate of 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] and was 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.

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

Test	Required reduction in antimicrobial treatment costs assuming samples per day, calculated from Warhurst *et al.*[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

Test	Required reduction in antimicrobial treatment costs assuming samples per day, calculated from Warhurst *et al.*[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

The machinery-purchasing requirements vary according to the machinery already available to 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)[150] compared with blood culture. Comparison of NMB is simple, and the strategy with the highest NMB is estimated to be the most cost-effective. *Figures 12–23* present findings that are combinations of the assumed mortality rate (either 13% or 29%), the maximum acceptable incremental cost-effectiveness ratio (MAICER) (either £20,000 or £30,000 per QALY gained) and the number of blood samples that need to be analysed per day (2.4, 17 or 68). It is likely that the mortality rate is inversely correlated with the number of tests per day, in that high 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.
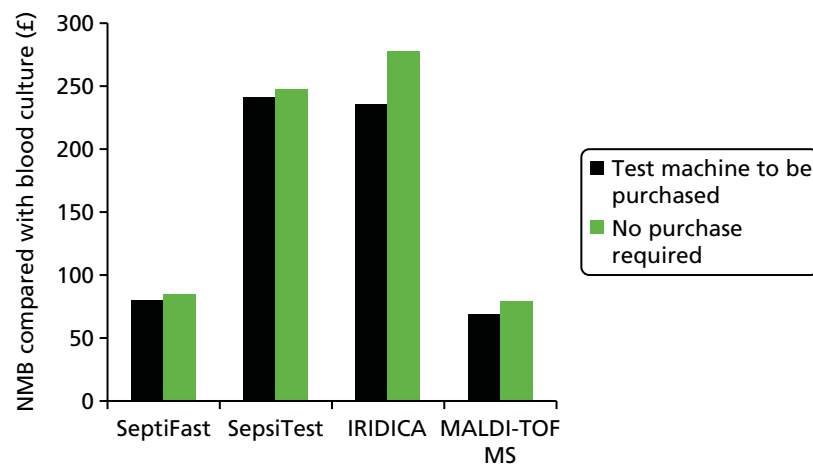
FIGURE 12 Net monetary benefit assuming a MAICER of £20,000, a mortality rate of 13% and 2.4 blood samples to be analysed per day.
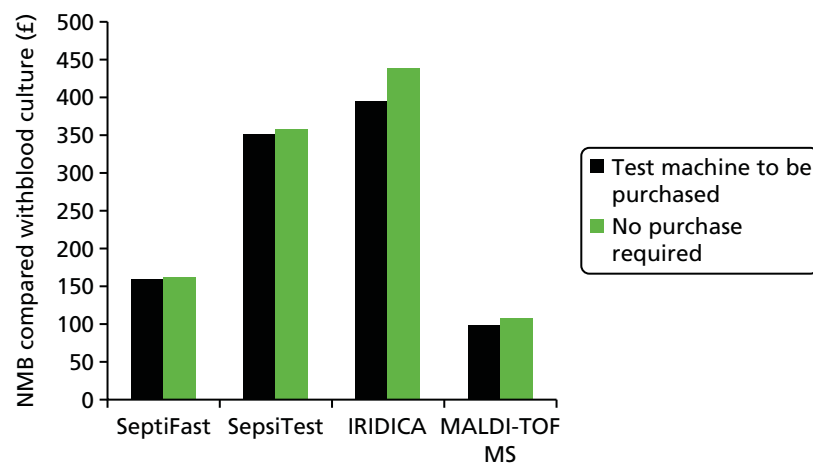
FIGURE 13 Net monetary benefit assuming a MAICER of £30,000, a mortality rate of 13% and 2.4 blood samples to be analysed per day.
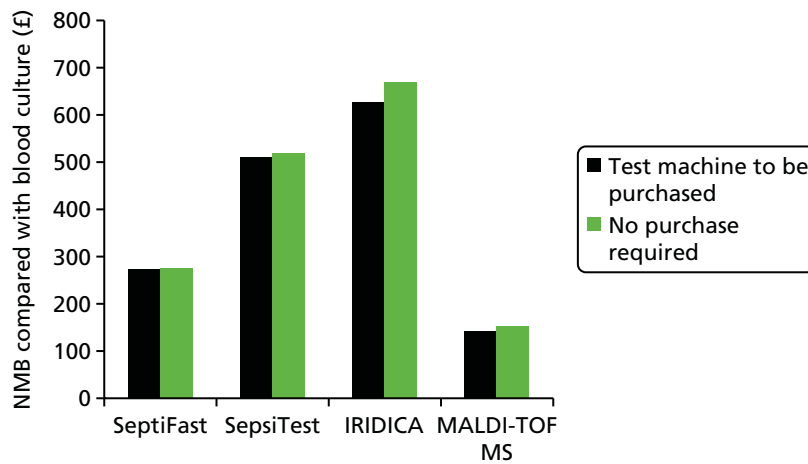
FIGURE 14 Net monetary benefit assuming a MAICER of £20,000, a mortality rate of 29% and 2.4 blood samples to be analysed per day.
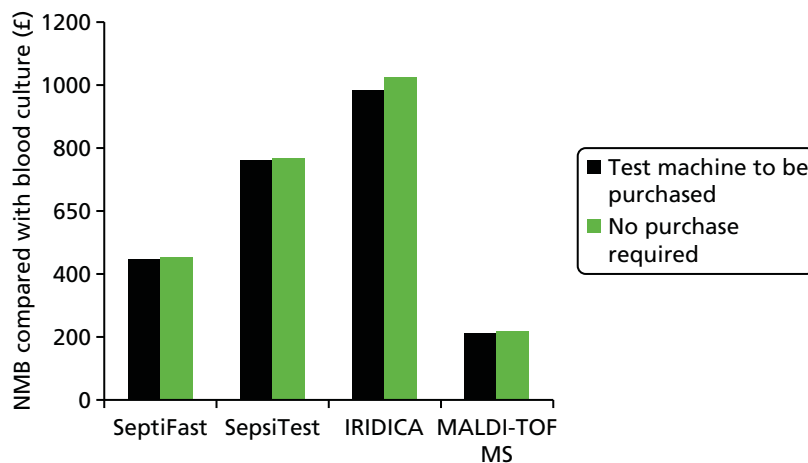
FIGURE 15 Net monetary benefit assuming a MAICER of £30,000, a mortality rate of 29% and 2.4 blood samples to be analysed per day.
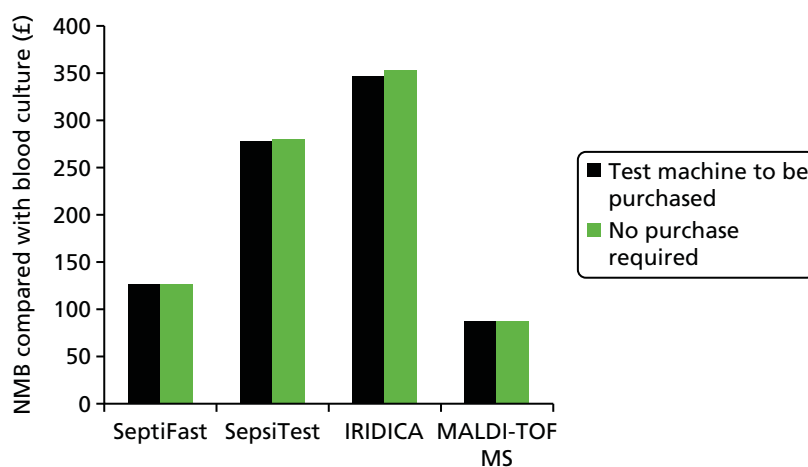
FIGURE 16 Net monetary benefit assuming a MAICER of £20,000, a mortality rate of 13% and 17 blood samples to be analysed per day.
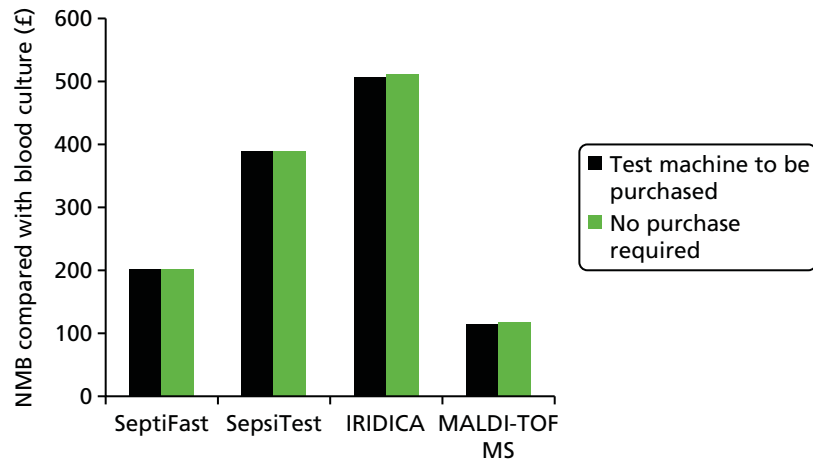
FIGURE 17 Net monetary benefit assuming a MAICER of £30,000, a mortality rate of 13% and 17 blood samples to be analysed per day.
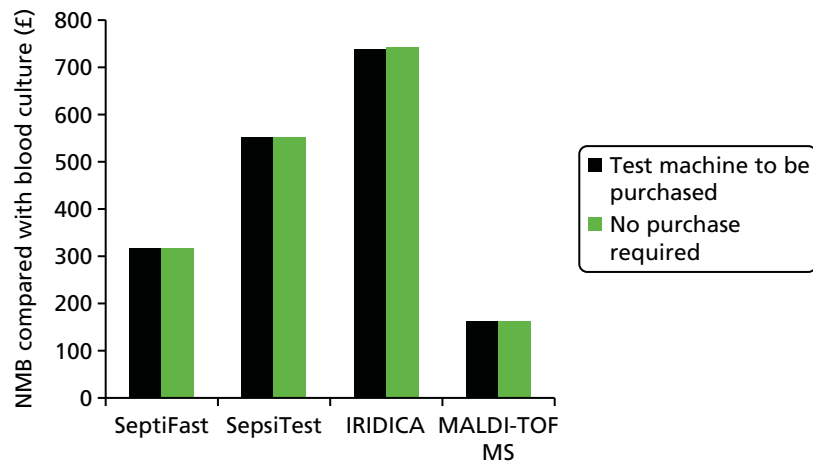
FIGURE 18 Net monetary benefit assuming a MAICER of £20,000, a mortality rate of 29% and 17 blood samples to be analysed per day.
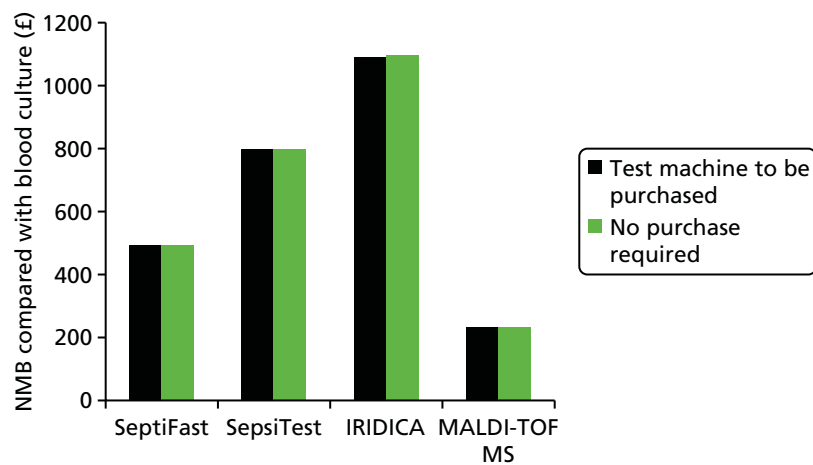
FIGURE 19 Net monetary benefit assuming a MAICER of £30,000, a mortality rate of 29% and 17 blood samples to be analysed per day.
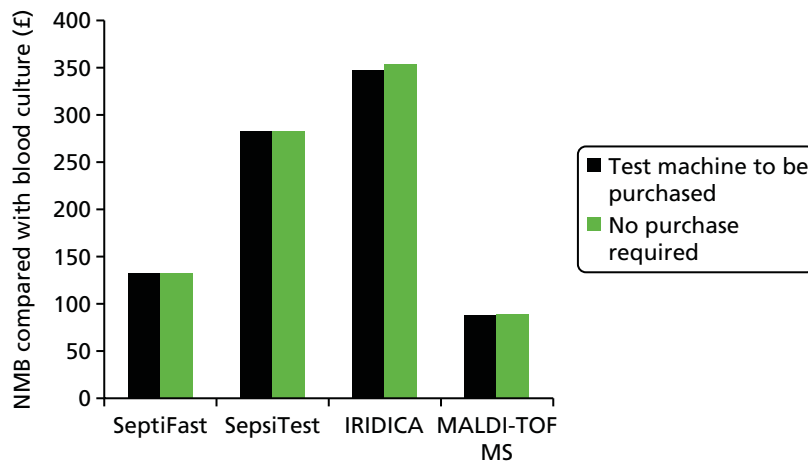
FIGURE 20 Net monetary benefit assuming a MAICER of £20,000, a mortality rate of 13% and 68 blood samples to be analysed per day.
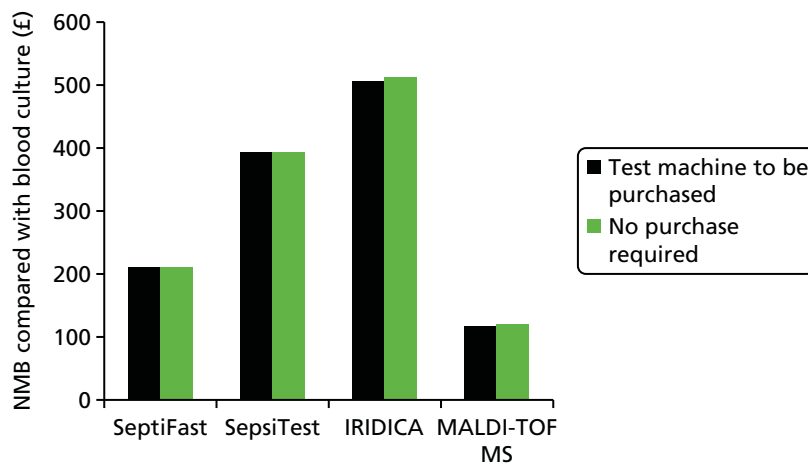
FIGURE 21 Net monetary benefit assuming a MAICER of £30,000, a mortality rate of 13% and 68 blood samples to be analysed per day.
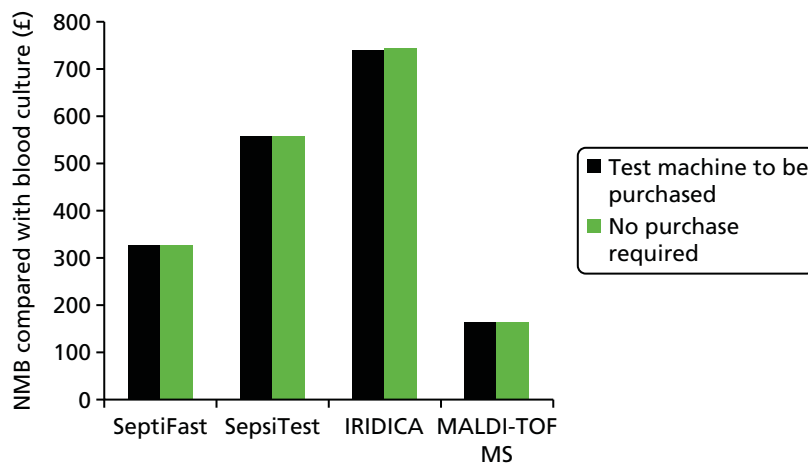
FIGURE 22 Net monetary benefit assuming a MAICER of £20,000, a mortality rate of 29% and 68 blood samples to be analysed per day.
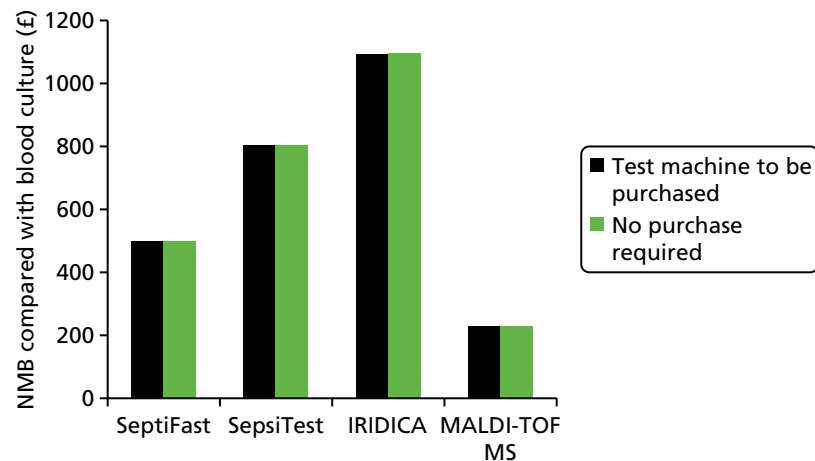
FIGURE 23 Net monetary benefit assuming a MAICER of £30,000, a mortality rate of 29% and 68 blood samples to be analysed per day.

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 or not the acquisition cost of the machine was incorporated in the calculation.

In the scenario in which the mortality rate is assumed to be 13%, the MAICER £20,000 per QALY and the number of blood samples per day 2.4, SepsiTest or IRIDICA has the highest estimated NMB depending on the 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 dependent on the individual clinician questioned and thus there is large uncertainty in these estimates.

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 needs to be purchased are shown in *Table 39*. The results when 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 7 days per week, 24 hours per day basis could be subsumed into the intervention costs while 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 (see *Table 39*): £201,782 + £100,000 = £301,782.

Incremental QALYs (see *Table 39*): 48.81.

ICER: £301,782/£48.81 = £6183.

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

Scenario and test	Incremental cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with blood culture[a]	Cost per QALY gained compared with blood culture (£)[b]
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

a These values include QALYs gained in subsequent years.
b Dominating means providing more QALYs for the same or lower cost.

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

Scenario and test	Incremental cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with blood culture[a]	Cost per QALY gained compared with blood culture (£)[b]
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

a These values include QALYs gained in subsequent years.
b Dominating means providing more QALYs for the same or lower cost.

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 needs to be purchased, are shown in *Table 41*. The results when no additional machinery needs to be purchased are provided in *Table 42*.

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] are the most appropriate, as these data were taken from a large study (853 patients) in an English population that was believed to be the study with the highest quality (see *Table 6*). The only study with a similar level of quality was that by Tafelski *et al.*[112] but, as that was a small study (88 patients) set in Germany, the results are believed to be not as generalisable as those from Warhurst *et al.*[10]

In an alternative scenario, the impact of using the estimated results from the synthesis of diagnostic accuracy data (see *Figure 3*) was explored. The mid-point 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 out of 922 (8.7%). These data result in an estimated positivity rate of 18.4% for SeptiFast with 30.6% of subsequent blood cultures also being positive and 67% being negative. In this analysis, a failure rate of 1.4% was assumed for SeptiFast, based on a naive pooling of failure results from Warhurst *et al.*[10] (69 failed samples) and from Paolucci *et al.*[99] (100 failed samples) divided by the number of samples analysed in the SeptiFast versus blood culture trials (11,659 samples).

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

Scenario and test	Incremental cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with blood culture[a]	Cost per QALY gained compared with blood culture (£)[b]
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

a These values include QALYs gained in subsequent years.
b Dominating means providing more QALYs for the same or lower cost.

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

Scenario and test	Incremental cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with blood culture[a]	Cost per QALY gained compared with blood culture (£)[b]
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

a These values include QALYs gained in subsequent years.
b Dominating means providing more QALYs for the same or lower cost.

The estimated cost per QALY values for SeptiFast, obtained using the results of the synthesis of diagnostic accuracy, are shown in *Table 43* (assuming a 13% mortality rate) and *Table 44* (assuming a 29% mortality rate).

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

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 concision, 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 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 and the same or fewer QALYs) to dominating (lower cost the same or more QALYs), indicating high levels of uncertainty in the assumed effectiveness of the interventions and MALDI-TOF MS.

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%

Scenario and test	Incremental cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with blood culture[a]	Cost per QALY gained compared with blood culture (£)[b]
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

a These values include discounted QALYs gained in subsequent years.
b Dominating means providing more QALYs for the same or lower cost.

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%

Scenario and test	Incremental cost per annum compared with blood culture (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with blood culture[a]	Cost per QALY gained compared with blood culture (£)[b]
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

a These values include discounted QALYs gained in subsequent years.
b Dominating means providing more QALYs for the same or lower cost.

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

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

Dominating means providing more or the same QALYs at a reduced cost.
Dominated means providing fewer or the same QALYs at an increased cost.

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

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

Dominating means providing more or the same QALYs at a reduced cost.
Dominated means providing fewer or the same QALYs at an increased cost.

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

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

Dominating means providing more or the same QALYs at a reduced cost.
Dominated means providing fewer or the same QALYs at an increased cost.

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

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

Dominating means providing more or the same QALYs at a reduced cost.
Dominated means providing fewer or the same QALYs at an increased cost.

Results from the threshold analyses

Threshold analyses are 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, 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 then 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.

Owing to the number of figures presented, the threshold analyses are contained in *Appendix 8*. Note that, in all analyses, the diagnostic accuracy for SeptiFast has been taken from Warhurst *et al.*,[10] as this was assumed to be a more representative study of English practice and was graded as a higher-quality study 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 to be analysed, are shown in *Table 49*. The values assuming that the comparator is MALDI-TOF MS are 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 gained of £20,000.

TABLE 49 Threshold levels require to achieve a cost per QALY gained of £20,000, assuming that the number of samples that need to be analysed per day is 2.4 and a comparator of blood culture

Test	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 that the number of samples that need to be analysed per day is 2.4 and a comparator of MALDI-TOF MS

Test	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

Results from the studies comparing SeptiFast with matrix-absorbed laser desorption/ionisation time-of-flight mass spectrometry and comparing SepsiTest with matrix-absorbed laser desorption/ionisation time-of-flight mass spectrometry

Two studies had a comparator of MALDI-TOF MS in addition to blood culture. These were the studies by Tafelski et al.,[112] in which the index test was SeptiFast, and by Loonen et al.,[114] in which the index text was SepsiTest. The cost-effectiveness of SeptiFast and SepsiTest was estimated using the data from the trials with MALDI-TOF MS as a comparator and assuming the average benefits estimated by the clinicians. These results were then compared 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 to see if they were concordant.

The benefits associated with the interventions were amended to account for any benefit associated with a positive MALDI-TOF MS. Thus, for example, if (as an illustrative example) SeptiFast was associated with an estimated reduction in ICU stay of 0.607 per positive test and MALDI-TOF MS with 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 $(0.607 - 0.175) \times 0.798$, 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. The 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 concision, evaluations in which machinery was not required to be purchased to use SeptiFast or SepsiTest are not reported. As no failures were mentioned by Tafelski et al.[112] or Loonen et al.,[114] it was assumed that there were none for the analyses presented.

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

Scenario and test	Incremental cost per annum compared with MALDI-TOF MS (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with MALDI-TOF MS[a]	Cost per QALY gained compared with MALDI-TOF MS (£)[b]
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

a These values include discounted QALYs gained in subsequent years.
b Dominating means providing more QALYs for the same or lower cost.

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

Scenario and test	Incremental cost per annum compared with MALDI-TOF MS (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year compared with MALDI-TOF MS[a]	Cost per QALY gained compared with MALDI-TOF MS (£)[b]
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

a These values include discounted QALYs gained in subsequent years.
b Dominating means providing more QALYS at the same or lower cost.

The results indicate that SeptiFast appears to be more cost-effective than MALDI-TOF MS when aggregated clinicians values are used. The results for SepsiTest are less conclusive, with estimated values of > £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, in which SepsiTest appeared considerably more cost-effective than both MALDI-TOF MS and SeptiFast (see *Figures 12–23*).

Results from studies comparing SeptiFast and SepsiTest simultaneously with blood culture

Two studies evaluated both SeptiFast and SepsiTest against blood culture: those by Schreiber and Nierhaus[119] and Leitner *et al.*[118] The cost-effectiveness of these studies was estimated to see if it was 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 to be zero. It was further assumed that machinery would need to be purchased for both tests. The ICERs for SeptiFast compared with SepsiTest are provided in *Table 53* (mortality rate assumed to be 13%) and in *Table 54* (mortality rate assumed to be 29%). It is seen that in all of the scenarios the ICER for SeptiFast compared with SepsiTest is > £30,000 per QALY gained. This conclusion is concordant with those of the main analyses as shown in *Figures 12–23*.

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	Test	Incremental cost per annum (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year[a]	Cost per QALY gained (£)[b]	Cost per QALY of SeptiFast compared with SepsiTest (£)[c]
Assuming 2.4 blood samples per day					
Schreiber and Nierhaus[119]	SeptiFast	87,022	5.81	14,975	90,855
	SepsiTest	49,147	5.39	9111	
Leitner *et al.*[118]	SeptiFast	67,686	6.95	9739	Dominated
	SepsiTest	−7910	9.27	Dominating	
Assuming 17 blood samples per day					
Schreiber and Nierhaus[119]	SeptiFast	337,533	41.21	8191	74,363
	SepsiTest	117,708	38.25	3077	
Leitner *et al.*[118]	SeptiFast	200,421	49.28	4067	Dominated
	SepsiTest	−286,890	65.73	Dominating	
Assuming 68 blood samples per day					
Schreiber and Nierhaus[119]	SeptiFast	1,195,262	164.83	7252	69,267
	SepsiTest	376,224	153.00	2459	
Leitner *et al.*[118]	SeptiFast	646,815	197.13	3281	Dominated
	SepsiTest	−1,242,168	262.91	Dominating	

a These values include QALYs gained in subsequent years.
b Dominating means providing more or the same QALYs at a reduced cost.
c Dominated means providing fewer or the same QALYs at an increased cost.

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	Test	Incremental cost per annum (£)	Incremental QALYs gained through number of avoided 30-day mortalities within 1 year[a]	Cost per QALY gained (£)[b]	Cost per QALY of SeptiFast compared with SepsiTest (£)[c]
Assuming 2.4 blood samples per day					
Schreiber and Nierhaus[119]	SeptiFast	87,022	12.96	6713	40,728
	SepsiTest	49,147	12.03	4084	
Leitner *et al.*[118]	SeptiFast	67,686	15.50	4366	Dominated
	SepsiTest	−7910	20.68	Dominating	
Assuming 17 blood samples per day					
Schreiber and Nierhaus[119]	SeptiFast	337,533	91.92	3672	33,335
	SepsiTest	117,708	85.33	1379	
Leitner *et al.*[118]	SeptiFast	200,421	109.94	1823	Dominated
	SepsiTest	−286,890	146.62	Dominating	
Assuming 68 blood samples per day					
Schreiber and Nierhaus[119]	SeptiFast	1,195,262	367.69	3251	31,051
	SepsiTest	376,224	341.31	1102	
Leitner *et al.*[118]	SeptiFast	646,815	439.75	1471	Dominated
	SepsiTest	−1,242,168	586.50	Dominating	

a These values include QALYs gained in subsequent years.
b Dominating means providing more or the same QALYs at a reduced cost.
c Dominated means providing fewer or the same QALYs at an increased cost.

Interpretation of the independent economic model results

Forming conclusions based on the economic modelling is very difficult because of 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, which includes only data from the published literature on patient-related outcomes, 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. The results contrasted with those in base case 1. Using the average values provided by the clinical experts, the estimated cost per QALY for all interventions was below a threshold of £20,000 per QALY in all scenarios. However, when the results were broken down by individual clinician estimates, there was a wide variation in the cost per QALY. In the scenario of a 13% mortality rate and 2.4 samples analysed per day, costs per QALY estimates were > £20,000 for four out of seven clinicians for SeptiFast, for two out of seven clinicians for SepsiTest, for four out of seven clinicians for IRIDICA and for two out of six 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 a direct comparison of the interventions, although, for completeness, these results have been presented along with a description of the key assumptions and data driving these comparative results. IRIDICA is estimated to have much better sensitivity than either SeptiFast or SepsiTest, and this results in an increase in QALYs and reduced costs resulting from reductions in ICU and hospital lengths of stay and changes in antimicrobial costs. The specificity of the interventions is similar, although the specificity of IRIDICA is marginally lower than that of SeptiFast or SepsiTest; this will result in 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 other tests. The QALY gains and cost savings associated with IRIDICA were sufficient to offset its higher costs and to confer on IRIDICA the highest NMB. The second highest NMB was typically for SepsiTest, not because of its inherent accuracy, as it has lower sensitivity than SeptiFast, but because the assumed cost per test is lower and the benefit per test estimated by the clinicians is higher. MALDI-TOF MS was presumed to be better than blood culture, but as the number of samples tested using MALDI-TOF MS was much smaller, with only those that were blood culture positive being tested, the NMB was lower than for any of the interventions.

Additional analyses undertaken using the results from multitest studies of SeptiFast, SepsiTest and blood culture and the data provided by clinicians, were concordant with base case 2, in that SeptiFast has an estimated cost per QALY gained value of > £20,000 compared with SepsiTest. However, the indirect results produced when using studies directly comparing MALDI-TOF MS were contradictory, 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 that 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 carried out, 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 comments that studies comparing the use of an intervention with standard practice, with the results of the tests 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. Although this study was recently completed, data analysis had not been fully conducted at the time of writing. When the results of the clinical effectiveness 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.

Chapter 4 Discussion

Statement of principal findings

Clinical effectiveness

A comprehensive systematic review and meta-analysis (where applicable) were undertaken to evaluate the clinical effectiveness of three interventions (SeptiFast, SepsiTest and IRIDICA) 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[44–46,48,49–65,67–88,90–99,101,102,104–119] of varying methodological quality were included. Most of these studies were considered to be at risk of bias and there are concerns about their applicability. Pooled effects for sensitivity and specificity across 54 studies[44–46,59–65,67–88,90–102,104–111,118,119] (comprising 10,010 patients) comparing SeptiFast with blood culture found that the specificity of SeptiFast (0.86, 95% CrI 0.84 to 0.89) is higher than its sensitivity (0.65, 95% CrI 0.60 to 0.71). Similarly, one study[112] that compared SeptiFast with blood culture plus MALDI-TOF MS found that SeptiFast had higher specificity (0.74, 95% CI 0.64 to 0.85) than sensitivity (0.58, 95% CI 0.30 to 0.86). However, because of the deficiencies in the quality of 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 metaregression for several potentially relevant characteristics including 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 were affected by these subgroups.

Pooled effects for sensitivity and specificity across four studies[48,113,118,119] (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 CrI is large. Comparison with blood culture plus MALDI-TOF MS in a single study[114] also showed higher specificity 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 because of the small number of studies included. Owing to the deficiencies in the quality of 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[49,115–117] [comprising 860 patients across two studies[115,117] (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), although 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 owing to the small number of studies included. Owing to the deficiencies in the quality of the included studies, the sensitivity and specificity data for IRIDICA may not be reliable and should be treated with caution.

Although 41 studies[44–46,59,60,64,66,68,69,72,74,76–78,80,81,84,85,88–90,92–94,96,97,99–101,103–107,110–112,114,116,117,119] across the three interventions reported data on one or more intermediate (e.g. 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 (e.g. 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,[100,110,112] all of SeptiFast, were identified. One did not investigate patient outcomes[112] and one was predominantly in febrile neutropenia patients and reported no significant difference in mortality, length of stay (ICU or hospital) or fever duration.[110] The third RCT was published in abstract form only and did not report a difference in mortality.[100]

Given the potentially fatal consequences of removing treatment from patients with sepsis, it is not anticipated that negative tests in isolation would be acted on in clinical practice were an intervention introduced. This is because none of the three tests has the very high sensitivity needed to be used as a 'rule-out' test, which would reassure clinicians that a negative result would be associated with a very low probability of a patient having sepsis. Therefore, the main advantage of the tests in patients who are receiving broad-spectrum antibiotics would be in providing an earlier indication of the pathogen than would be achieved by standard methods, and in the potential focusing of antibiotic treatments. If negative tests are not acted upon, then the numbers of false-positive tests are reduced in populations with a high incidence of sepsis, such as patients in the ICU. It may be that the benefits of the tests are greater in high-incidence populations.

In addition, the three interventions provide very limited data regarding antimicrobial sensitivity. Definitive data on this are needed, to be obtained if possible, using standard culture methods undertaken in parallel with the interventions.

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[103] and one not.[89] The remaining two presented results from modelling studies, with one evaluating SeptiFast[66] and one evaluating an IRIDICA–PLEX-ID hybrid.[128] The External Assessment Group noted limitations of 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 clinicians 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 < £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 among the individual clinicians.

Additional analyses, using the data provided by clinicians, were undertaken to assess whether or not 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 > £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 in the number of days in the ICU and reduced antimicrobial costs.

Strengths and limitations of the assessment

Clinical effectiveness

The strengths of this systematic review are that it was conducted using robust methods, including the development of a prespecified 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 metaregression and parameter estimates were produced using Markov chain Monte Carlo simulation.

The assessment of methodological quality was generally hampered by the poor quality of reporting in the included SeptiFast, SepsiTest and IRIDICA studies, with the majority of studies being classified as being 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 is 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.[10,12] As a result, the reported estimates of sensitivity and specificity are likely to be biased (underestimated) compared with 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-analyses 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.,[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.[151] 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. That review included a number of studies that were excluded from the present review because of publication type (foreign language, n = 2) or because they did not meet our inclusion criterion of 'suspected sepsis' (n = 2). In addition, Chang et al.[151] pooled studies comparing SeptiFast results against various reference standards to produce composite overall diagnostic accuracy metrics. These factors may have contributed to the the fact that diagnostic performance metrics were higher than those found in the present review and by Dark et al.[50]

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 that provide rapid information on bloodstream 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 few 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 is severely limited given the feedback from clinicians regarding the difficulty of the task and also because of 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 is expected to influence the conclusion that until further research is performed 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 hours a day, 7 days 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.

Uncertainties

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 few 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.

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 on, 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.

Chapter 5 Conclusion

Implications for service provision

Given the considerable uncertainty in the cost-effectiveness results produced for each intervention, it is uncertain what the implications in the NHS would entail. Were the interventions deemed to be a cost-effective use of resources, it is likely that reconfigurations of working practice, such as moving to a 7-day working schedule, would be required in order that the interventions could provide results more quickly than under the present system.

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 better reported, in accordance to the standards for the reporting of diagnostic accuracy studies statement.[152] Once robust diagnostic accuracy data have been established, there is a need for a pragmatic trial in which the results from the interventions are allowed to change patient management and for these results to be compared with standard practice in order to allow robust estimates of the clinical effectiveness 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 few 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.

Conclusions

Clinical effectiveness

SeptiFast, SepsiTest and IRIDICA appear to have higher specificity values than sensitivity values. However, because of 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 few 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 *Research recommendations*).

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 because of the limitations of the evidence base. The studies recommended in *Research recommendations* would reduce this uncertainty. Threshold analyses have been provided that may allow decision-makers to estimate whether or not the interventions are likely to meet a level at which the decision-makers would consider the interventions to be cost-effective.

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Contributions of authors

Data sharing statement

Data can be obtained from the corresponding author, subject to their being non-confidential.

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

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

Search strategy

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?	'Yes' if states consecutive or random 'No' if states another method of patient sampling/selection 'Unclear' if unclear or not reported	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
Was a case–control design avoided?	'Yes' 'No' 'Unclear' if insufficient information provided	
Did the study avoid inappropriate exclusions?	'Yes' if the study provides explicit exclusion criteria and appropriately select participants that are typical of patients with bloodstream 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 bloodstream infection/suspected sepsis) 'Unclear' if insufficient information provided	
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?	'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	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
Were the reference standard results interpreted without knowledge of the results of the index test?	'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	

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	Could the patient flow have introduced bias?
	'No' if reference standard and index tests not performed on blood samples drawn at different times	'Low risk' if all domains are 'yes' 'High risk' if one or more domain is 'no'
	'Unclear' if insufficient information is provided	'Unclear risk' anything in between
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	
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 or not 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 bloodstream infection/suspected sepsis
		'No' if characteristics of participants are well described and typical of patients with bloodstream 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, for example 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

Reference details	Reason for exclusion
Clinicaltrials.gov. *Benefit of SeptiFast Multiplex PCR in the Etiologic Diagnosis and Therapeutic Approach for Onco-Hematology Patients Presenting Sepsis*. 2010. URL: http://ClinicalTrials.gov/show/NCT00561639 (accessed April 2016)	Trial record with no study results
Clinicaltrials.gov. *Diagnosis of Septicaemia by Detection of Microbial DNA in Blood in Severe Infections*. 2011. URL: http://ClinicalTrials.gov/show/NCT00709358 (accessed April 2016)	Protocol: SeptiFast EVAMICA trial
Clinicaltrials.gov. *Value of the LightCycler© SeptiFast Test MGRADE for the Pathogen Detection in Neutropenic Hematological Patients*. 2012. URL: http://ClinicalTrials.gov/show/NCT01114165 (accessed April 2016)	Trial record of Idelevich et al.[a]
Clinicaltrials.gov. *Evaluation in the Treatment of Nosocomial Sepsis Comparing Polymerase Chain Reaction with Conventional Blood Culture*. 2013. URL: http://ClinicalTrials.gov/show/NCT01450358 (accessed April 2016)	Replaced (protocol) by full-text paper reported by Rodrigues et al.[b]
Clinicaltrials.gov. *Optimal Antibiotic Treatment of Moderate to Severe Bacterial Infections*. 2014. URL: http://ClinicalTrials.gov/show/NCT01338116 (accessed April 2016)	Ongoing SeptiFast trial – estimated completion January 2017
Abbott Molecular Inc. *IRIDICA BAC BSI Assay – Package Insert Ref: 08N22-010*. Des Plaines, IL: Abbott Molecular Inc.; 2014	Replaced (package insert) by full-text paper reported by Metzgar et al.[c]
Afsharpaiman S, Mamishi S, Pourakbari B, Siyadati A, Tabatabaee P, Khotaee G. Diagnosis of bacteremia using universal PCR in febrile ill children. *Acta Med Iranica* 2007;**45**:131–8	Cultured samples (positive)
Al-Zahrani AKH, Ghonaim MM, Hussein YM, Eed EM, Khalifa AS, Dorgham LS, *et al.* Evaluation of recent methods versus conventional methods for diagnosis of early-onset neonatal sepsis. *J Infect Develop Countries* 2015;**9**:388–93	Not intervention (test) of interest
Arabestani MR, Fazzeli H, Nasr Esfahani B. Identification of the most common pathogenic bacteria in patients with suspected sepsis by multiplex PCR. *J Infect Develop Countries* 2014;**8**:461–8	Cultured samples (positive)
Avolio M, Diamante P, Zamparo S, Modolo M, Grosso S, Zigante P, *et al.* Molecular identification of bloodstream pathogens in patients presenting to the emergency department with suspected sepsis. *Shock* 2010;**34**:27–30	Diagnostic metrics data included in Avolio et al.[d] (confirmed by authors)
Avolio M, Diamante P, Zamparo S, Modolo ML, Grosso S, Zigante P, *et al.* Evaluation of molecular detection of bloodstream pathogens in 144 patients arriving in the emergency room with clinical signs of sepsis. *Clin Microbiol Infect* 2010;**16**:S533–4	Replaced (abstract) by full-text paper reported by Avolio et al.[e]
Baraki H, Al AA, Schilling T, Pichlmaier M, Martens A, Haverich A, *et al.* Are universal rRNA gene PCR and sequencing tests an alternative to conventional culture analysis for infected alloplastic implants? *Thorac Cardiovasc Surg* 2012;**60**:114	Specimens from tissue samples only
Bauer KA, West JE, Balada-Llasat JM, Pancholi P, Stevenson KB, Goff DA. An antimicrobial stewardship program's impact. *Clin Infec Dis* 2010;**51**:1074–80	Cultured samples (positive)
Bernaschi P, Ranno S, Lucignano B, Pizzorno B, Liesenfeld O, Menichella D. Value of multiplex-PCR (SeptiFast) for the diagnosis of bacterial and fungal pathogens in newborns and children with suspected sepsis. *Clin Microbiol Infect* 2010;**16**:S541	Replaced (abstract) by full-text paper reported by Lucignano et al.[f]

TABLE 55 Studies excluded from the clinical review (*continued*)

Reference details	Reason for exclusion
Bilkovski R, Sampath R, Huiras M, Durtschi A, Ecker D, Chalfin D, *et al. The Potential Clinical and Economic Value of Rapid Diagnosis of Suspected Bloodstream Infections using PCR/ESI-MS*. 54th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 5–9 September 2014	Replaced (abstract/poster) by full-text paper reported by Vincent *et al.*[9]
Bingold T, Just L, Roenneberg T, Sartorius S, Hunfeld K, Wissing H. Septifast allows more rapid detection of liver transplant patients being at risk of sepsis than blood culture or inflammatory parameters. *Infection* 2009;**37**:40	Not target population (patients scheduled for orthotopic liver transplant)
Brealey D, Libert N, Pugin J, Chalfin D, Sampath R, Ecker D, *et al. RADICAL Study: Rapid Diagnosis of Suspected Bloodstream Infections using PCR/ESI-MS*. 54th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 5–9 September 2014	Replaced (abstract/poster) by full-text paper reported by Vincent *et al.*[9]
Burdino E, Milia M, Milano R, Gregori G, Allice T, Ruggiero T, *et al.* Evaluation of the SEPTIFAST real-time PCR for rapid identification of blood pathogens in patients with suspected sepsis: an experience in a northwestern Italy hospital. *Clin Microbiol Infect* 2012;**18**:505	Replaced (abstract) by full-text paper reported by Burdino *et al.*[h]
Cambau E, Courcol R, Veerabudun V, Bretagne S, Durand-Zaleski I, Bastuji-Garin S, *et al. Does Performing DNA Detection in Blood Improves the Microbial Diagnosis of Severe Bloodstream Infections? First Results of the EVAMICA study.* Proceedings of the 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin, Germany, 27–30 April 2013	Insufficient information to allow calculation of diagnostic 2 × 2 table (SeptiFast EVAMICA trial)
Casalta JP, Gouriet F, Roux V, Thuny F, Habib G, Raoult D. Evaluation of the LightCycler SeptiFast test in the rapid etiologic diagnostic of infectious endocarditis. *Eur J Clin Microbiol* 2009;**28**:569–73	Not target population (patients with infective endocarditis)
Chaidaroglou A, Manoli E, Gkouziouta A, Gourzi P, Pantou M, Saroglou G, *et al.* Clinical impact of a multiplex real-time PCR assay (SeptiFast) for the rapid detection of pathogens in patients with end-stage heart failure bridged to heart transplantation with ventricular assist devices. *Crit Care* 2010;**14**:S13	Not target population (implantable ventricular-assist device patients suspected of infection)
Chaidaroglou A, Manoli E, Gkouziouta A, Aggelaki D, Pantou M, Saroglou G, *et al.* The use of a multiplex real-time PCR assay for the detection of bacterial and fungal bloodstream infections in thoracic allograft recipients. *J Heart Lung Transpl* 2011;**30**(Suppl. 1):S146	Not target population (thoracic allograft recipients)
Chaidaroglou A, Manoli E, Gkouziouta A, Kolovou V, Gourzi P, Pandou M, *et al.* The contribution of a multiplex real-time PCR assay (SeptiFast) for the rapid detection of bacterial and fungal bloodstream infections in patients with end-stage heart failure bridged to heart transplantation with ventricular assist devices. *Transplantation* 2012;**94**:945	Not target population (implantable ventricular-assist device patients suspected of infection)
Chaidaroglou A, Manoli E, Gkouziouta A, Gourzi P, Pantou M, Kolovou V, *et al.* The contribution of a multiplex real-time PCR to detect bacterial and fungal bloodstream infections in a cohort of thoracic allograft recipients. *Transplantation* 2012;**94**:77	Not target population (thoracic allograft recipients)
Chaidaroglou A, Manoli E, Marathias E, Gkouziouta A, Saroglou G, Alivizatos P, *et al.* Use of a multiplex polymerase chain reaction system for enhanced bloodstream pathogen detection in thoracic transplantation. *J Heart Lung Transpl* 2013;**32**:707–13	Not target population (thoracic allograft recipients)
Chan KYY, Lam HS, Cheung HM, Chan AKC, Li K, Fok TF, *et al.* Rapid identification and differentiation of Gram-negative and Gram-positive bacterial bloodstream infections by quantitative polymerase chain reaction in preterm infants. *Crit Care Med* 2009;**37**:2441–7	Cultured samples (positive/negative)
Clerici D, Mancini N, Forno B, Cappelli B, Mastaglio S, Messina C, *et al.* Molecular diagnosis by lightcycler? SeptiFast in the preemptive treatment of invasive fungal infections: a 5 cases report. *Haematologica* 2009;**94**:415	Insufficient information to allow calculation of diagnostic 2 × 2 table
Conen A, Schaub N, Achermann Y, Battegay M, Frei R, Trampuz A. Is multiplex PCR (SeptiFast) useful for diagnosis of infectious endocarditis? *Clin Microbiol Infect* 2009;**15**:S531–2	Not target population (patients with suspected native or prosthetic valve infective endocarditis)

TABLE 55 Studies excluded from the clinical review (*continued*)

Reference details	Reason for exclusion
Dark P, Dunn G, Chadwick P, Young D, Bentley A, Carlson G, *et al.* The clinical diagnostic accuracy of rapid detection of healthcare-associated bloodstream infection in intensive care using multipathogen real-time PCR technology. *BMJ Open* 2011;**1**:e000181	Replaced (protocol) by full-text paper reported by Warhurst *et al.*[i]
Diamante P, Avolio M, Zamparo S, Grosso S, Tosoni N, Zigante P, *et al.* [Molecular diagnosis of sepsis: the experience at the Pordenone hospital.] *Riv Ital Medi Lab* 2010;**6**:205–10	Foreign language (Italian)
Disqué C, Kochem AJ, Mühl H, Lorenz MG, Sakka SG. PCR detection of sepsis-inducing pathogens in blood using SepsiTest™. *Int J Med Microbiol* 2008;**298**:7	No outcome data
Disqué C, Sakka S, Wellinghausen N. Klinische Evaluation von SepsiTest™ zum universellen PCR-Nachweis von bakteriellen Erregern in Vollblut. *Der Mikrobiologe Heft 1* 2010;**20**:13–16	Foreign language (German)
Disqué C, Mühl H, Gebert S, Winter J, Matten J, Wellinghausen N. Microbe detection in whole blood without blood culture. *J Mol Diagn* 2010;**12**:882	Replaced (abstract) by full-text paper reported by Wellinghausen *et al.*[j]
Disqué C, Gebert S, Kochem AJ, Mühl H, Matten J, Sakka SG, *et al. A Multicentre Study of Bacteraemia using a New Commercial Universal 16S rDNA PCR Test*. 8th World Congress on Trauma, Shock, Inflammation and Sepsis, Munich, Germany, 9–13 March 2010	Replaced (abstract) by full-text paper reported by Wellinghausen *et al.*[j]
Disqué C, Mühl H, Keim S, Lorenz MG. Automated extraction of microbial DNA from whole blood for the universal PCR detection of pathogens. *Infection* 2011;**39**:S115–16	No comparator (study investigating the influence of blood volume)
Disqué C, Linow M, Murphy N. Broad-range microbial DNA isolation from clinical specimens for universal PCR diagnosis. *J Mol Diagn* 2012;**14**:687	Specimens (liquid and tissue) from different body sites and mixed population
Disqué C, Keim S, Mühl H, Lorenz MG. DNA extraction from broad range of micro-organisms for molecular diagnosis. *Clin Microbiol Infect* 2012;**18**:775	Specimens (liquid and tissue) from different body sites and mixed population)
Draz NI, Taha SE, Abou Shady NM, Abdel Ghany YS. Comparison of broad range 16S rDNA PCR to conventional blood culture for diagnosis of sepsis in the newborn. *Egypt J Med Hum Genet* 2013;**14**:403–11	Not intervention (test) of interest
Dubská L, Vyskočilová M, Minaríková D, Jelínek P, Tejkalová R, *et al.* LightCycler SeptiFast technology in patients with solid malignancies: clinical utility for rapid etiologic diagnosis of sepsis. *Crit Care* 2012;**16**:404	Not target population (patients with solid malignancy)
Elwan AE, Zarouk WA. Diagnosis of neonatal bacterial sepsis by polymerase chain reaction. *J Biol Sci* 2009;**9**:533–40	Not intervention (test) of interest
Enomoto M, Morioka I, Morisawa T, Yokoyama N, Matsuo M. A novel diagnostic tool for detecting neonatal infections using multiplex polymerase chain reaction. *Neonatology* 2009;**96**:102–8	Not intervention (test) of interest
Gosiewski T, Jurkiewicz-Badacz D, Sroka A, Brzychczy-Wloch M, Bulanda M. A novel, nested, multiplex, real-time PCR for detection of bacteria and fungi in blood. *BMC Microbiol* 2014;**14**:144	Not intervention (test) of interest
Greco R, Clerici D, Mancini N, Clementi M, Lorentino F, Crucitti L, *et al.* Multiplex PCR-based assay (SeptiFast) for rapid detection of pathogens in febrile neutropenia: results in 273 consecutive patients. *Bone Marrow Transplant* 2012;**47**:S77–8	Replaced (abstract) by full-text paper reported by Barbanti *et al.*[k]
Greco R, Mancini N, Lorentino F, Crucitti L, Barbanti C, Forcina A, *et al.* Rapid molecular detection of pathogens in 1941 blood samples from 516 consecutive patients with febrile neutropenia. *Bone Marrow Transplant* 2014;**49**:S334	Insufficient information to allow calculation of diagnostic 2 × 2 table
Grif K, Heller I. Improvement of detection of bacterial pathogens in normally sterile body sites with a focus on orthopedic samples by use of a commercial 16S rRNA broad-range PCR and sequence analysis. *J Clin Microbiol* 2012;**50**:2250–4	Specimens (liquid and tissue) from different body sites

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TABLE 55 Studies excluded from the clinical review (*continued*)

Reference details	Reason for exclusion
Haag H, Locher F, Nolte O. Molecular diagnosis of microbial aetiologies using SepsiTest™ in the daily routine of a diagnostic laboratory. *Diagn Microbiol Infect Dis* 2013;**76**:413–18	Specimens (liquid and tissue) from different body sites
Halasz E, Petro M, Bojtos I, Myszoglad R, Simon J. Rapid diagnosis of sepsis with molecular biological methods. *Clin Chem Lab Med* 2012;**50**:eA32–3	Insufficient information to allow calculation of diagnostic 2 × 2 table (not a diagnostic study)
Halliday CL, Sorrell TC, Chen SCA. Detection of multiple fungal species in blood samples by real-time PCR: an interpretative challenge. *J Clin Microbiol* 2014;**52**:3515–16	Letter/comment with no details of intervention
Hettwer S, Wilhelm J, Hammer D, Schürmann M, Amoury M, Scheubel S, *et al.* Sepsis in the emergency department: pathogen identification by blood cultures and PCR. *Crit Care* 2009;**13**:S154	Insufficient information to allow calculation of diagnostic 2 × 2 table
Holmes C, Kirk-Granger H, Perera N. *The Clinical Usefulness of Multiplex PCR for the Detection of Bacteria and Fungi in the Blood of Patients with Haematological Malignancies*. Proceedings of the 24th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain, 10–13 May 2014	Not target population (haematological malignancies)
Horvath A, Peto Z, Urban E, Vagvolgyi C, Somogyvari F. A novel, multiplex, real-time PCR-based approach for the detection of the commonly occurring pathogenic fungi and bacteria. *BMC Microbiol* 2013;**13**:300	Not intervention (test) of interest
Idelevich EA, Niederbracht Y, Tafelski S, Nachtigall I, Berdel WE, Peters G, *et al.* Clinical value of the SeptiFast Multiplex PCR test in hematologic patients with neutropenic fever or sepsis: interim study results. *Int J Med Microbiol* 2011;**301**:7	Replaced (abstract) by full-text paper reported by Idelevich *et al.*[l]
Irwin AD, Barton T, Grant A, Williams R, Carrol ED. SepsiTest molecular diagnosis of bacteraemia in febrile paediatric patients. *Clin Microbiol Infect* 2012;**18**:505	Not target population (patients with increased levels of C-reactive protein)
Jordan JA, Durso MB, Butchko AR, Jones JG, Brozanski BS, Durso MB, *et al.* Evaluating the near-term infant for early onset sepsis: progress and challenges to consider with 16S rDNA polymerase chain reaction testing. *J Mol Diagn* 2006;**8**:357–63	Not intervention (test) of interest
Jordana-Lluch E, Carolan HE, Giménez M, Sampath R, Ecker DJ, Quesada D, *et al.* Rapid diagnosis of bloodstream infections with PCR followed by mass spectrometry. *PLOS ONE* 2013;**8**:e62108	Not intervention (test) of interest (used older extraction method on PLEX-ID system, thus not comparable to the current IRIDICA platform)
Josefson P, Stralin K, Ohlin A, Ennefors T, Dragsten B, Andersson L, *et al.* Evaluation of a commercial multiplex PCR (SeptiFast) in the aetiological diagnosis of community-acquired bloodstream infections. *Clin Microbiol Infect* 2010;**16**:S541–2	Replaced (abstract) by full-text paper reported by Josefson *et al.*[m]
Kalenka A, Timm J, Schmid S, Beck G. Value of LightCycler SeptiFast in detection of ventilator-associated pneumonia. *Intens Care Med* 2009;**35**:S197	Not target population (patients with suspected ventilator associated pneumonia)
Kalenka A, Schmid S, Timm J, Beck G. Lightcycler SeptiFast as a tool to enhance the detection of bacteremia and fungemia in patients with intraabdominal infection during antimicrobiological therapy. *Intens Care Med* 2009;**35**:S196	No comparator (i.e. not vs. blood culture)
Kaleta EJ, Clark AE, Johnson DR, Gamage DC, Wysocki VH, Cherkaoui A, *et al.* Use of PCR coupled with electrospray ionization mass spectrometry for rapid identification of bacterial and yeast bloodstream pathogens from blood culture bottles. *J Clin Microbiol* 2011;**49**:345–53	Cultured samples (positive)
Karam El Din ZA, Mokhtar E, El-Shereef A, Abd El KA, Al-Tounisy A. Rapid diagnosis of neonatal sepsis caused by yeast infection. *Mycoses* 2012;**55**:75	Not intervention (test) of interest
Kim B, Park S, Kim T, Kim J, Rim D, Choi T, *et al.* [Clinical efficacy evaluation of multi-parameter realtime polymerase chain reaction for the central venous catheter-related blood stream infection.] *Infect Chemother* 2011;**43**:240–4	Foreign language (Korean)

TABLE 55 Studies excluded from the clinical review (*continued*)

Reference details	Reason for exclusion
Kuhn C, Disqué C, Mühl H, Orszag P, Stiesch M, Haverich A. Molecular diagnosis of the etiological agents of infectious endocarditis using commercial universal rRNA gene PCR plus sequencing tests. *Infection* 2011;**39**:S124–25	Replaced (abstract) by full-text paper reported by Kuhn *et al.*[n]
Kuhn C, Disqué C, Mühl H, Orszag P, Stiesch M, Haverich A. Evaluation of commercial universal rRNA gene PCR plus sequencing tests for identification of bacteria and fungi associated with infectious endocarditis. *J Clin Microbiol* 2011;**49**:2919–23	Not target population (patients with suspected infectious endocarditis and used valvular and blood samples for analysis)
Lefort A, Chartier L, Sendid B, Wolff M, Mainardi J, Podglajen I, *et al.* Diagnosis, management and outcome of *Candida endocarditis*. *Clin Microbiol Infect* 2012;**18**:E99–109	Not target population (patients with *Candida* endocarditis)
Lehmann LE, Alvarez J, Hunfeld KP, Goglio AP, Kost GJ, Louie RF, *et al.* Model analysis of clinical utility of PCR in microbiological testing for sepsis. *Infection* 2009;**37**:44	Not intervention (test) of interest
Leli C, Cardaccia A, D'Alo F, Ferri C, Bistoni F, Mencacci A. A prediction model for real-time PCR results in blood samples from febrile patients with suspected sepsis. *J Med Microbiol* 2014;**63**:649–58	Study aim to develop prediction model from positive SepsitFast results only
Liberto MC, Puccio R. Applications of LightCycler *Staphylococcus* M-GRADE assay to detect *Staphylococcus aureus* and coagulase-negative staphylococci in clinical blood samples and in blood culture bottles. *Infezioni in Medicina* 2006;**14**:71–6	Not intervention (test) of interest
Liu CL, Ai HW, Wang WP, Chen L, Hu HB, Ye T, *et al.* Comparison of 16S rRNA gene PCR and blood culture for diagnosis of neonatal sepsis. *Arch Pediatrie* 2014;**21**:162–9	Not intervention (test) of interest
Lodes U, Lippert H. [Molecular biological sepsis diagnostic using multiplex PCR in surgical intensive care as suitable alternative to conventional microbial culture – a representative overview.] *Zentralbl Chir* 2011;**136**:135–42	Foreign language (German)
Markota A, Golle A, Sinkovic A. Polymerase chain reaction analysis in patients with sepsis. *Intens Care Med* 2013;**39**:S453	Diagnostics metrics data (from patients and samples) included in a full-text study by Markota *et al.*[o]
Martinez MDM, Arredondo AR, Alvarez EM, Prieto AMP, Caballero MP, Mari JMN. Lightcycler septifast trading system in molecular microbiological diagnosis of neonatal sepsis. *J Matern-Fetal Neonat Med* 2010;**23**(Suppl. 1):156	Coagulase-negative staphylococci detection only
Mencacci A, Leli C, Montagna P, Cardaccia A, Meucci M, Bietolini C, *et al.* Diagnosis of infective endocarditis: comparison of the LightCycler SeptiFast real-time PCR with blood culture. *J Med Microbiol* 2012;**61**:881–3	Not target population (patients with suspected infective endocarditis)
Mencacci A, Leli C, Cardaccia A, Meucci M, Moretti A, D'Alo F, *et al.* Procalcitonin predicts real-time PCR results in blood samples from patients with suspected sepsis. *PLOS ONE* 2012;**7**:e53279	Insufficient information to allow calculation of diagnostic 2 × 2 table
Merisescu M, Luminos M, Jugulete G, Florea D, Streinu-Cercel A. Molecular diagnosis of severe bacterial sepsis in children. *Crit Care* 2012;**16**:67	Cultured samples (blood and/or fluids)
Merisescu M, Jugulete G, Streinu-Cercel A, Luminos M. Plex Id role in the diagnosis of acute bacterial sepsis with *E-coli* in children. *Pediatr Crit Care Med* 2014;**15**(Suppl. 1):150–1	*Klebsiella pneumoniae* detection only (no details on sample type, comparator methods or useable outcome data)
Meyer T, Franke G, Polywka S, Lutgehetmann M, Gbadamosi J, Magnus T, *et al.* Improved detection of bacterial central nervous system infections by use of a broad-range PCR assay. *J Clin Microbiol* 2014;**52**:1751–3	Specimens from cerebrospinal fluid samples
Molina JM, Cordoba J, Ramirez P, Gobernado M. [Automatic detection of bacterial and fungal infections in blood.] *Enferm Infect Microbiol Clin* 2008;**26**(Suppl. 9):75–80	Foreign language (Spanish)
Mongelli G, Romeo MA, Denaro C, Gennaro M, Fraggetta F, Stefani S. An added value of multi-pathogen probe-based real-time PCR SeptiFast in the rapid diagnosis of bloodstream infections in patients with bacteremia. *J Med Microbiol* 2015;**64**:670–5	Not target population (febrile patients with suspected bacteraemia)

TABLE 55 Studies excluded from the clinical review (*continued*)

Reference details	Reason for exclusion
Moore MS, McCann CD, McCarroll M, May L, Jordan JA. Direct detection of bacteria from blood of ED and ICU patients being evaluated for bloodstream infection. *J Mol Diagn* 2014;**16**:727	Not intervention (test) of interest
Mundy L, Hiller JE. *Rapid Molecular Assays for the Diagnosis of Sepsis and Identification of Sepsis Causing Pathogens*. Adelaide, SA: Adelaide Health Technology Assessment (AHTA). Horizon Scanning Prioritising Summary. 2010. URL: www.horizonscanning.gov.au/internet/horizon/publishing.nsf/Content/C8A5BA60BD01A93ECA257757000A2015/$File/Volume_27_June_2010_sepsis.pdf (accessed April 2016)	Review
Niederbracht Y, Idelevich EA, Penner H, Berdel WE, Peters G, Silling G, *et al.* Applicability of a commercial multiplex PCR test for identification of true blood stream infections with coagulase-negative staphylococci in neutropenic hematological patients. *Int J Med Microbiol* 2013;**303**:13	Coagulase-negative staphylococci detection only
Nieman A, Rozemeijer W, de Jong E, Beishuizen B, van Agtmael M, Savelkoul P, *et al.* Molecular detection of bacterial bloodstream infections: validation of the SepsiTest™ assay. *Ned Tijdschr Med Microbiol* 2010;**18**:109	Preclinical validation study
Nieman A, De Jong E, Beishuizen B, Koek A, Savelkoul PH, Schade RP. Molecular detection of bacterial bloodstream infections: the SepsiTest assay. *Clin Microbiol Infect* 2011;**17**:S729	Insufficient information to allow calculation of diagnostic 2 × 2 table
Novak-Frazer L, Rautemaa-Richardson R, Denning D, Bowyer P. Multiplex PCR for the rapid diagnosis of skin/soft tissue and bloodstream infections. *Clin Microbiol Infect* 2012;**18**:122–3	Specimens from whole blood and wound swabs (and insufficient information to allow calculation of diagnostic 2 × 2 table)
Ohlin A, Backman A, Ewald U, Schollin J, Bjorkqvist M. Diagnosis of neonatal sepsis by broad-range 16S real-time polymerase chain reaction. *Neonatology* 2012;**101**:241–6	Not intervention (test) of interest
Orszag P, Disqué C, Keim S, Lorenz M, Wiesner O, Hadem J, *et al.* Monitoring of patients supported by extracorporeal membrane oxygenation for systemic infections by broad-range rRNA gene PCR amplification and sequence analysis. *J Clin Microbiol* 2013;**52**:307–11	Not target population (patients supported by extracorporeal membrane oxygenation)
Ortiz Ibarra F, Reyna J, Trevino P, Fernandez L, Lara G, Valenzuela E, *et al.* A standardized protocol for the multiplex PCR technique Septifast Roche for neonatal samples with suspected sepsis. *Crit Care* 2012;**16**:79	Insufficient information to allow calculation of diagnostic 2 × 2 table
Palomares JC, Puche B, Martos A, Lucena F, Marin M, Martin-Mazuelos E. Rapid molecular diagnosis of severe sepsis in patients with SIRS. *Clin Microbiol Infect* 2009;**15**:S529–30	Replaced (abstract) by full-text paper reported by Palomares *et al.*[p]
Pleskova T, Greplova K, Bacikova L, Dubska L. What is the added value of molecular microbiology in sepsis? *Clin Chem Lab Med* 2011;**49**:S610	Cultured samples (positive) and unclear if cancer patients have sepsis
Popov DA, Vostrikova TI. [The first experience of application of PCR techniques in real-time mode to diagnose bacteriemia during postoperational period in cardiosurgery patients.] *Klin Labor Diagn* 2011;**8**:49–52	Foreign language (Russian)
Raineri SM, Canzio D, Sarno C, Cascio ND, Mineo G, Chiaramonte R, *et al.* LightCycler SeptiFast in early diagnosis of sepsis: our experience. *Crit Care* 2009;**13**:S153	Insufficient information to allow calculation of diagnostic 2 × 2 table
Ratanarat R, Cazzavillan S, Ricci Z, Rassu M, Segala C, de Cal M, *et al.* Usefulness of a molecular strategy for the detection of bacterial DNA in patients with severe sepsis undergoing continuous renal replacement therapy. *Blood Purificat* 2007;**25**:106–11	Not intervention (test) of interest
Reier-Nilsen T, Farstad T, Nakstad B, Lauvrak V, Steinbakk M. Comparison of broad range 16S rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: a case control study. *BMC Pediatr* 2009;**9**:5	Not intervention (test) of interest

TABLE 55 Studies excluded from the clinical review (*continued*)

Reference details	Reason for exclusion
Rogina P, Skvarc MM, Stubljar DD. Diagnostic utility of broad range bacterial 16S rRNA gene PCR with degradation of human and free bacterial DNA in bloodstream infection is more sensitive than an in-house developed PCR without degradation of human and free bacterial DNA. *Mediators Inflamm* 2014;**2014**:108592	Insufficient information to allow calculation of diagnostic 2 × 2 table
Sahre H, Vogel S, Park JW, Weber S. [Sepsis-diagnostics with help of SeptiFast®-systems: experience on a internal intensive care unit.] *Infection* 2007;**35**:9–10	Foreign language (German)
Sakka S, Wellinghausen N, Kochem AJ, Disqué C, Muehl H, Gebert S, *et al.* A multicentre study of bacteraemia using a new commercial universal 16S rDNA PCR test. *Inflamm Res* 2010;**59**:s137	Replaced (abstract) by full-text paper reported by Wellinghausen *et al.*[j]
Sampath R. Advanced techniques for detection and identification of viral contaminants using the Ibis PLEX-ID universal biosensor. *Pda J Pharm Sci Tech* 2011;**65**:690	Conference abstract not available
Santolaya ME, Farfan MJ, De La Maza, Farfan MJ. Diagnosis of bacteremia in febrile neutropenic episodes in children with cancer: microbiologic and molecular approach. *Pediatr Infect Dis J* 2011;**30**:957–61	Not intervention (test) of interest
Schaub N, Boldanova T, Noveanu M, Arenja N, Hermann H, Twerenbold R, *et al.* Incremental value of multiplex real-time PCR for the early diagnosis of sepsis in the emergency department. *Swiss Med Wkly* 2014;**144**:w13911	Diagnostic metrics data included in Schaub *et al.*[q]
Shaat SS, El Shazly SA, Badr Eldin MM, Barakat SS, Hashish MH. Role of polymerase chain reaction as an early diagnostic tool for neonatal bacterial sepsis. *J Egypt Public Health Assoc* 2013;**88**:160–4	Not intervention (test) of interest
Sitnik R, Marra A, Petroni R, Ramos O, Martino M, Pasternak J, *et al.* SeptiFast for diagnosis of sepsis in severely ill patients from a Brazilian Hospital. *J Med Diagn* 2011;**13**:736	Replaced (abstract) by full-text paper reported by Sitnik *et al.*[r]
Skvarc M, Stublar D, Rogina P. Broad-range 16S rRNA gene PCR using SepsiTest in conjunction with valid clinical data and sepsis biomarkers improve sepsis diagnostics. *Int J Med Microbiol* 2012;**302**:6	Insufficient information to allow calculation of diagnostic 2 × 2 table
Steinmann J, Buer J, Paul A, Saner F, Rath PM. Diagnostic performance of multiplex PCR for diagnosis of bloodstream infections in liver transplant recipients with suspected sepsis. *Int J Med Microbiol* 2011;**301**:51	Replaced (abstract) by full-text paper reported by Rath *et al.*[s]
Stubljar D, Rogina P, Skvarc M, Pavlovic A. *Diagnostic Accuracy of sCD14 (Presepsin) is Comparable to Procalcitonin (PCT) for the Diagnosis of Bacterial Infections in Critically Ill Patients*. Proceedings of the 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin, Germany, 27–30 April 2013	Not intervention (test) of interest
Tafelski S, Nachtigall I, Idelevich E, Silling G, Becker K, Faust J, *et al. Impact of Septi-Fast for Pathogen Detection in Critical-Care Patients with Sepsis: Results from a Randomized Controlled Clinical Trial*. Proceedings of the 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin, Germany, 27–30 April 2013	Replaced (abstract) by full-text paper reported by Tafelski *et al.*[t]
Torres-Martos E, Pérez-Ruiz M, Pedrosa-Corral I, Peña-Caballero M, Jiménez-Valera MM, Pérez-Ramirez MD, *et al.* [Evaluation of the LightCycler SeptiFast test in newborns and infants with clinical suspicion of sepsis.] *Enferm Infecc Microbiol Clin* 2013;**31**:375–9	Foreign language (Spanish)
Tsalik EL, Jones D, Nicholson B, Caram LB, Liesenfeld O, Fowler VG, *et al.* Detection of bacterial and fungal pathogens associated with sepsis in patients presenting to the emergency room. *Intens Care Med* 2009;**35**:S196	Insufficient information to allow calculation of diagnostic 2 × 2 table
Vrsajkov V, Pejakovic J, Dragic Z, Radanovic B. Pathogen identification in septic patients: clinical importance of PCR and blood cultures. *Intens Care Med* 2014;**40**(Suppl. 1):259	Not intervention (test) of interest
Warhurst G, Dunn G, Chadwick P, Blackwood B, McAuley D, Perkins GD, *et al.* Rapid detection of health-care-associated bloodstream infection in critical care using multipathogen real-time polymerase chain reaction technology: a diagnostic accuracy study and systematic review. *Health Technol Assess* 2015;**19**(35)	Replaced (Health Technology Assessment monograph) by full-text journal paper reported by Warhurst *et al.*[i]

TABLE 55 Studies excluded from the clinical review (*continued*)

Reference details	Reason for exclusion
Zerweck A, Raepple D, Finck A, Finke J, Bertz H. PCR-based molecular diagnostic tool (Septifast) in septic patients undergoing induction therapy or stem cell transplantation in haematological malignancies. *Bone Marrow Transplant* 2010;**45**:S228	Not target condition (patients undergoing induction therapy and or stem cell transplantation)
Ziegler I, Josefson P, Olcen P, Molling P, Stralin K. Quantitative data from the SeptiFast real-time PCR is associated with disease severity in patients with sepsis. *BMC Infect Dis* 2014;**14**:155	Diagnostic metrics data (secondary analysis) included in Josefson *et al.*[m]

a Idelevich EA, Niederbracht Y, Tafelski S, Nachtigall I, Berdel WE, Peters G, *et al.* Clinical Value of the SeptiFast Multiplex PCR test in hematologic patients with neutropenic fever or sepsis: interim study results. *Int J Med Microbiol* 2011;**301**:7.

b Rodrigues C, Dos Santos MS, Filho HHC, Charbel CE, da Silva LCS, Rossi F, *et al.* Rapid molecular test (SeptiFast) reduced time for adjustment of antibiotic treatment in comparison with conventional blood cultures in critically ill sepsis patients: a randomized controlled clinical trial (preliminary results). *Crit Care* 2013;**17**:P26.

c Metzgar D, Frinder M, Rothman R, Peterson S, Carroll KC, Zhang S, *et al.* Ibis Biosciences, 2015 (unpublished).

d Avolio M, Diamante P, Modolo M, De Rosa R, Stano P, Camporese A. Direct molecular detection of pathogens in blood as specific rule-in diagnostic biomarker in patients with presumed sepsis: our experience on a heterogeneous cohort of patients with signs of infective systemic inflammatory response syndrome. *Shock* 2014;**42**:86–92.

e Avolio M, Diamante P, Zamparo S, Modolo M, Grosso S, Zigante P, *et al.* Molecular identification of bloodstream pathogens in patients presenting to the emergency department with suspected sepsis. *Shock* 2010;**34**:27–30.

f Lucignano B, Ranno S., Liesenfeld O, Pizzorno B, Putignani L, Bernaschi P, *et al.* Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. *J Clin Microbiol* 2011;**49**:2252–8.

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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	Inclusion criteria (illness aetiology)	Exclusion criteria
Single index test studies: SeptiFast					
Dierkes et al. (2009)[64] 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)[44] 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 included	NR
Bingold et al. (2007)[59] 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	Inclusion criteria (illness aetiology)	Exclusion criteria
Klemm et al. (2007)[60] 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 beats per minute), tachypnoea (> 20 breaths per minute) or hyperventilation ($PaCO_2 <$ 33 mmHg)] were patients included	NR
Lodes et al. (2008)[61] 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 included	NR
Louie et al. (2008)[46] USA Roche Diagnostics and a grant from the National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health 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

TABLE 56 Study characteristics (*continued*)

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	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 73 (70.9%) samples were drawn from heavily neutropenic patients	NR
Vince et al. (2008)[62] 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	ICU (n = 17), outside ICU (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 included	NR
Dark et al. (2009)[63] 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 bloodstream infection were included	NR
Gimeno et al. (2009)[65] Spain, single centre Sponsor/funding: NR Abstract	Single gate Prospective Sampling method: NR 5 months (dates NR)	NR	NR	Haematological 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	Inclusion criteria (illness aetiology)	Exclusion criteria
Lehmann et al. (2009)[66]	Single gate	ICU, emergency room, medical and surgical wards	Emergency department, in hospital and intensive/critical care	Included patients were aged ≥ 18 years, suspected sepsis, a blood culture drawn and subsequent antibiotic treatment initiation or change	NR
Germany, five centres	Retrospective study				
Supported, in part, by Roche Diagnostics	Sampling consecutive				
Full text	Sampling period: NR				
Lodes et al. (2009)[67]	Single gate	Surgical ICU	Intensive/critical care	Surgical patients with SIRS and subsequent need of intensive care were included	NR
Germany	Prospective study				
Sponsor/funding: NR	Consecutive sample				
Full text	4 months (May–August 2006)				
Palomares et al. (2009)[68]	Single gate	ICU	Intensive/critical care	Reported that patients with suspected bloodstream infection and two SIRS were included	NR
Spain, single centre	Study type: NR				
Sponsor/funding: NR	Sampling method: NR				
Abstract	Sampling period: NR				
Paolucci et al. (2009)[69]	Single gate	NR	NR	Reported that patients included were newborns aged > 3 days with late-onset sepsis	NR
Italy	Retrospective study				
Sponsor/funding: NR	Sampling method: NR				
Correspondence	Sampling period: NR				

TABLE 56 Study characteristics (*continued*)

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Varani et al. (2009)[70] 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 included	NR
von Lilienfeld-Toal et al. (2009)[71] 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 (September 2001–February 2002; April 2003–January 2004)	Tertiary care hospital haematology ward	In hospital	NR (included patients with febrile neutropenia after chemotherapy for haematological malignancies)	NR
Westh et al. (2009)[72] Germany, multicentre (n = 6) Research funding from Roche Diagnostics Full text	Single gate Study type: NR Sampling method: NR 5 months (June–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)[73] Austria Sponsor/funding: NR Abstract	Single gate Study type: NR Sampling method: NR Sampling period: NR	Neonatology	Neonatal unit	Very low-birthweight infants	NR

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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	Inclusion criteria (illness aetiology)	Exclusion criteria
Bloos et al. (2010)[74] Germany, France Roche Full text	Single gate Prospective study Sampling method: NR December 2005–April 2007	Intensive care	Intensive/critical care	Presence of severe sepsis or septic shock according to the ACCP/SCCM consensus criteria	Exclusion criteria: aged <18 years or previous enrolment in this trial
Lamoth et al. (2010)[75] Switzerland, single centre Roche Diagnostics Full text	Single gate Prospective Consecutive 14 months (September 2006–November 2007)	University hospital isolation ward	In hospital	NR (included febrile neutropenic adult haematological patients undergoing induction or consolidation chemotherapy for acute leukaemia or autologous haematopoietic stem cell transplantation)	NR
Lehmann et al. (2010)[76] Germany, two centres Research funding, reagents, and equipment from Roche Diagnostics Full text	Single gate Prospective study Sampling method: NR Sampling period: NR	Surgical ICUs	Intensive/critical care	Reported that all adult patients who were clinically suspected of suffering from severe sepsis of bacterial or fungal origin were included. Inclusion followed after independent decision of the physician in charge to call for a blood culture	NR
Maubon et al. (2010)[77] 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 were included	NR

TABLE 56 Study characteristics (*continued*)

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Regueiro et al. (2010)[78] 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–May 2008)	Intensive care and anaesthesiology services	In hospital and intensive/critical care	Reported that patients who all met criteria for SIRS and suspected sepsis on admission were included	NR
Soki et al. (2010)[79] Hungary, single centre Sponsor/funding: NR Abstract	Single gate Study type: NR Sampling method: NR Sampling period: NR	ICU and haematology department	In hospital and intensive/critical care	Reported that patients displaying symptoms of sepsis with or without antibiotic therapy were included	NR
Tsalik et al. (2010)[80] USA, two centres National Institutes of Health grant and Roche Full text	Single gate Study type: NR Sampling method: NR 66 months	University medical centre emergency department (trauma centre) and Veterans Affairs Medical Centre	Emergency department	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 SIRS within a 24-hour period	Patients were excluded if they were aged < 18 years, if they had an imminently terminal comorbid condition or if they were participating in an ongoing clinical trial. Only subjects admitted to the hospital and for whom blood culture results were available were included in this analysis

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Wallet et al. (2010)[81] 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 °C) or hypothermia (≤36 °C) were eligible	NR
Yanagihara et al. (2010)[82] 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–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 included	NR
Bravo et al. (2011)[83] Spain Sponsor/funding: NR Full text	Single gate Study type: NR Non-consecutive sample 8 months (February–September 2009)	Medical and surgical ICU	In hospital and intensive/critical care	Development of a febrile episode in neutropenic or ICU patients that required hospital admission, or occurred during hospital stay	Receipt of empirical antibiotic treatment prior to blood sampling for analysis
Hettwer et al. (2011)[84] Germany Roche Full text	Single gate Prospective study Sampling method: NR August 2006–March 2009	Emergency department	Emergency department	Reported that patients aged > 18 years and admitted with clinical signs prompting physician to draw blood culture were included	NR

TABLE 56 Study characteristics *(continued)*

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Josefson *et al.* (2011)[85] 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–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 included	Patients with HIV and with hepatitis B and C infections
Lucignano *et al.* (2011)[86] Italy, single centre Sponsor/funding: NR Full text	Single gate Retrospective study Sampling method: NR 26 months	ICUs and surgery; oncology, haematology and neonatology; emergency department and paediatrics	In hospital and intensive/critical care	Reported that patients with clinical suggestion of 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 were included	NR
Obara *et al.* (2011)[87] Japan Partly funded by Roche Full text	Single gate Study type: NR Consecutive sample 6 months (September 2004–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 SIRS were 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	Inclusion criteria (illness aetiology)	Exclusion criteria
Vrioni et al. (2011)[88] 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 ICU were included	NR
Alvarez et al. (2012)[89] Spain Sponsor/funding: NR Full text	Single gate Retrospective study Sampling method: NR (cost-minimisation study) 12 months	ICU	ICU	Patients with a diagnosis of severe sepsis or septic shock were included	NR
Grif et al. (2012)[90] Austria Pfizer Full text	Single gate Prospective study Consecutive sample 3 months (January 2009–March 2009)	ICU and general wards	In hospital and intensive/critical care	Reported that patients with presumed sepsis (> 2 SIRS criteria) were included	NR
Guido et al. (2012)[91] Italy Sponsor/funding: NR Full text	Single gate Study type: NR Consecutive sample 12 months (January 2010–December 2010)	Haematology department	In hospital and unclear if intensive/ critical care	Reported that patients with febrile neutropenia (temperature > 38.0 °C) and neutrophil count < 0.5 × 10⁹/L in presence of acute and/or chronic blood disorders or bone marrow transplant were included	NR

TABLE 56 Study characteristics (*continued*)

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Lodes *et al.* (2012)[92] 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 in ICU with clinical diagnosis of sepsis within last 24 hours at risk of abdominal sepsis with non-threatening SIRS were included	NR
Mauro *et al.* (2012)[93] 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 <1 × 10³/µl), exposure to immunosuppressive agents, haematological malignancy, or solid tumour, were included	NR
Pasqualini *et al.* (2012)[94] 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 suspected of having SIRS caused by bacterial or fungal infection, and for whom blood culture was performed for causative pathogen identification were included	NR
Rath *et al.* (2012)[95] Germany, single centre Sponsor/funding: NR Full text	Single gate Prospective study Sampling method: NR 24 months (May 2009–April 2011)	Department of General, Visceral and Transplant Surgery	Intensive/critical care	Reported that ICU patients with suspected sepsis according to the criteria of the ACCP/SCCM were 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	Inclusion criteria (illness aetiology)	Exclusion criteria
Tschiedel et al. (2012)[96] Germany, multicentre Sponsor/funding: NR Full text	Single gate Retrospective study Sampling method: NR 19 months (May 2009–December 2010)	Different hospitals	In hospital and intensive/critical care	Reported that critically ill patients with symptoms of systemic infection were included	NR
Herne et al. (2013)[97] Estonia Sponsor/funding: NR Full text	Single gate Retrospective study Sampling method: NR March 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 were included. Patients with clinically proven sepsis or septic shock, or severe infection without known etiologic agent, were included. Patients with only blood culture or SeptiFast collected samples were included	NR
Kasper and Altiok (2013)[98] 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-birthweight infants when infection was suspected after 72 hours of life were included)	NR
Paolucci et al. (2013)[99] Italy, single centre The University of Bologna, the Italian Ministry of Education, University and Research (MIUR) Full text	Single gate Prospective Consecutive 22 months (June 2008–March 2010)	Haematology and the Paediatric Oncology and Haematology Unit, St Orsola-Malpighi University Hospital, Bologna, Italy	In hospital	NR (included severely neutropenic with haematological malignancies)	NR

TABLE 56 Study characteristics (*continued*)

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Rodrigues et al. (2013)[100] Brazil Sponsor/funding: NR Abstract	Single gate Prospective RCT Sampling method: NR 7 months	NR (patients from cardiology hospital)	NR	Included adult patients aged > 18 years staying > 48 hours in hospital, with clinical suspicion of sepsis	NR
Avolio et al. (2014)[101] Italy, single centre Sponsor/funding: NR Full text	Single gate Prospective study Consecutive sample 3 years (September 2008–December 2011)	Emergency and ICU	Emergency department and intensive/critical care	Patients aged > 18 years, admitted to the emergency department of the Santa Maria degli Angeli Hospital (Pordenone, Italy) with suspected bloodstream infections and at least two criteria of SIRS, were included	NR
Burdino et al. (2014)[102] Italy Sponsor/funding: NR Full text	Single gate Study type: NR Sampling method: NR October 2008–December 2012	Samples from infectious disease, ICU, cardiology, internal and surgical departments	In hospital and intensive/critical care	Reported that patients who had signs and symptoms of sepsis as defined by SIRS with suspected bacterial or fungal infections were included	NR
Mancini et al. (2014)[103] Italy, two 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 were 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	Inclusion criteria (illness aetiology)	Exclusion criteria
Markota et al. (2014)[104] Slovenia, single centre Sponsor/funding: NR Full text	Single gate Prospective study Sampling method: NR 13 months (September 2011–September 2012)	Medical ICU	Intensive/critical care	Specific inclusion criteria NR (adults who fulfilled the criteria for severe sepsis or septic shock were included)	NR
Ozkaya-Parlakay et al. (2014)[105] Turkey, single centre Sponsor/funding: NR Full text	Single gate Prospective study Consecutive sample 30 months (September 2009–February 2012)	Paediatrics department, university hospital	NR	Reported that patients included were aged between 1 month and 17 years without immune deficiency	NR
Schaub et al. (2014)[106] Switzerland, single centre Research grants from the University of Basel, the Department of Internal Medicine and Roche Full text	Single gate Prospective study Consecutive sample 20 months (June 2007–January 2009)	University hospital	Emergency department	Patients presenting to the emergency department with suspected sepsis were included. Both patients with and without prior antimicrobial therapy were included	Patients aged < 18 years
Sitnik et al. (2014)[107] Brazil, multicentre (n = 2) Roche Diagnostics donated all material multiplex PCR testing and training on test workflow Full text	Single gate Prospective study Consecutive sample 11 months (December 2008–October 2009)	ICU, emergency room and oncology patients	Intensive/critical care (and oncology patients)	Reported that all patients with infection plus two or more SIRS were included	NR

TABLE 56 Study characteristics (*continued*)

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Barbanti *et al.* (2015)[108] Italy, single centre Sponsor/funding: NR Abstract	Single gate NR Consecutive Months NR (2009–13)	Haematology and bone marrow transplant unit	In hospital	Haematological patients with febrile neutropenia were included	NR
Calitri *et al.* (2015)[109] Italy, single centre None Full text	Single gate Retrospective Sampling method: NR 37 months (September 2009–September 2012)	Wards (various), ICU	In hospital and intensive/critical care	Paediatric patients with suspected sepsis, febrile neutropenia, fever without focus or localised infective focus were included	NR
Idelevich *et al.* (2015)[110] Germany, single centre Partly funded by Roche Diagnostics and Pfizer Full text	Single gate Prospective RCT Sampling method: NR 28 months (May 2010–September 2012)	University Hospital Münster	NR	Patients who developed febrile neutropenia according to IDSA criteria were included. 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

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Tafelski et al. (2015)[112]

Germany, multicentre

Roche Deutschland

Full text | RCT

Randomised, double-blind, parallel-group trial

Consecutive sample

20 months (August 2010–March 2012) | ICUs | 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 defined as suspected or proven infection causing systemic inflammation with at least two of the following:

(a) leucocyte count <4 or >12/nl
(b) body temperature <36 °C or fever >38 °C
(c) tachypnoea >20 breaths per minute or hyperventilation ($PaCO_2$ <32 mmHg)
(d) tachycardia >90 beats per minute

Infections were defined by the treating physicians using standardised criteria for infection management, implemented on the participating wards since 2006. All patients were included only once, even if they had multiple episodes of sepsis during their ICU stay. Additionally, infection onset was required to be <72 hours to reduce the risk of detecting persistent circulating DNA. Additional criteria (because of limited availability of trained staff to undertake PCR testing during evening and weekends) to ensure an adequate comparison, patients were only enrolled in the study between 6 p.m. and 6 a.m. because PCR tests were available for only 12 hours | Exclusion criteria were patients aged < 18 years, pregnant, in police custody, missing or withdrawn informed consent, or participating in another prospective clinical study |

TABLE 56 Study characteristics (*continued*)

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Warhurst *et al.* (2015)[111] UK, multicentre (*n* = 4) UK National Institute for Health Research Health Technology Assessment programme Full text	Single gate Prospective study Consecutive sample 2 years 6 months (30 July 2010–31 January 2013)	Critical care	Intensive/critical care	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 hours of hospital admission or recent exposure to hospital care were included. Suspicion of bloodstream infection was based on the development of two or more SIRS criteria. Patient inclusion was then based entirely on the clinical decision to perform urgent blood culture investigations	Patients already recruited into the study, except where a subsequent, new episode of suspected health-care-associated bloodstream infection had developed, and/or patients placed on an end-of-life care pathway were excluded
Single index test studies: SepsiTest					
Wellinghausen *et al.* (2009)[48] Germany, multicentre Bundesministerium für Wirtschaft und Technologie Full text	Single gate Prospective Sampling method: NR 11 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) were included	NR
Loonen *et al.* (2014)[114] The 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 (November–December 2011 and October–December 2012)	Hospital emergency department	Emergency department	Reported that patients with two or more SIRS criteria and clinical signs of infection presenting at the emergency department were 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	Inclusion criteria (illness aetiology)	Exclusion criteria
Nieman et al. (2015)[113] Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed
Single index test studies – IRIDICA					
Bacconi et al. (2014)[49] USA, single centre Sponsor/funding: NR but majority authors are employees of Ibis Biosciences (an Abbott company) Full text	Single gate Prospective study Sampling method: NR 4 months (January–April 2012)	Hospital emergency department	Emergency department	Subjects were considered eligible if they were aged ≥ 18 years, were having blood cultures drawn as part of clinical care and were able to provide informed consent	NR
Delco-Volante et al. (2015)[115] Country: NR Abbott Conference presentation	Single gate Prospective study Consecutive sample 17 months (August 2013–December 2014)	NR	NR	NR [patients included neonates (aged <28 days) if the treating physicians diagnosed a suspected sepsis and intended to treat them with antibiotics]	NR
Vincent et al. (2015)[116] Belgium, UK, Switzerland, France, Poland, Germany Ibis Biosciences Inc., Abbott Full text	Single gate Study type: NR Sampling method: NR 8 months (October 2013–June 2014)	ICU	Intensive/critical care	Patients were considered for inclusion if they had (1) suspected or proven severe infection or sepsis; or (2) suspected or proven health-care-associated pneumonia, ventilator-associated pneumonia or severe community-acquired pneumonia	Patients were excluded if the treating clinician expected the patient to be discharged from the ICU on the day of evaluation or the following day, the treatment intent was palliative, the clinician was not committed to aggressive treatment or death was deemed imminent and inevitable

TABLE 56 Study characteristics (*continued*)

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	Inclusion criteria (illness aetiology)	Exclusion criteria
Metzgar et al. (2015)[117] Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed
Two index test studies: SeptiFast and SepsiTest					
Leitner et al. (2013)[118] Austria 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 included	NR
Schreiber and Nierhaus (2013)[119] 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–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

ACCP, American College of Chest Physicians; HIV, human immunodeficiency virus; IDSA, Infectious Diseases Society of America; IL-6, interleukin 6; LBP, lipopolysaccharide-binding protein; NR, not reported; $PaCO_2$, partial pressure of carbon dioxide; PCT, procalcitonin; SCCM, Society of Critical Care Medicine.

TABLE 57 Population characteristics

Single index test studies: SeptiFast

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Dierkes et al. (2009)[64]	Adults; aged 55 years (median) 49/77 (64%) male 35 (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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 77 patients analysed 99 paired samples
Raglio et al. (2006)[44]	Adults/children/neonates: NR; age, NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 74 patients analysed 114 paired samples
Bingold et al. (2007)[59]	Adults/children/neonates: not stated; age: NR Number male: NR Number 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[153]	Site of infection: NR Other symptoms: NR	21 patients included Number of patients not followed up: NR Samples from 21 patients analysed 134 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Klemm *et al.* (2007)[60]	Adults/children/neonates: NR; age: NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 44 patients analysed 56 paired samples
Lodes *et al.* (2008)[61]	Adults/children/neonates: NR; age: NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 137 patients analysed 358 paired samples
Louie *et al.* (2008)[46]	Adults; aged > 18 years [male, median 47 (range 18–80) years; female, median 46 (range 18–91) years] 122/200 (61%) male AIDS, immunosuppressant, 8 (4%) Antibiotics prior to blood sample: NR Previous antibiotic exposure: NR	Septic shock/MODS, 2 (1%)	Suspected bloodstream infection and at least two criteria of the SIRS criteria	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 Number of patients not followed up: NR Samples from 200 patients analysed 200 paired samples

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Mancini et al. (2008)[45]	Adults; aged 47 (range 21–69) years 23/34 (67.6%) male Number 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, n (%): 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 Number of patients not followed up: NR Samples from 34 patients analysed 103 paired samples
Vince et al. (2008)[62]	Adults/children/neonates: NR; age: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 36 patients analysed 39 paired samples
Dark et al. (2009)[63]	Adults; age: NR Number male: NR Number 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 Number of patients not followed up: NR Samples from 50 patients analysed 90 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Gimeno et al. (2009)[65]	Adults/children: NR; age: NR Number male: NR Number immunocompromised: NR Antibiotics prior to blood sample 19/19 (100%). All patients received antibiotics and/or antifungal prophylaxis Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease: NR	NR	Site of infection: NR Other symptoms: NR	Number of patients recruited: NR Number of patients not followed up: NR 19 patients analysed 45 paired blood samples
Lehmann et al. (2009)[66]	Adults; mean age 54.8 (range 18–92) years 268/436 (61%) male Number immunocompromised: NR Antibiotics prior to blood sample: NR Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease: 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	Number of patients recruited: NR 436 patients with 467 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; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Lodes et al. (2009)[67]	Adults (range: NR); ages 60.5 (14.7) years 30/52 (58%) male Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 52 patients analysed 258 paired samples
Palomares et al. (2009)[68]	Adults; age: NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 73 patients analysed 76 paired samples
Paolucci et al. (2009)[69]	Neonates aged > 3 days; mean age NR (however, newborns were aged > 3 days) Number male: NR One patient was affected by primary congenital immunodeficiency Antibiotics prior to blood sample: NR (however, three blood cultures – 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 > 2.0 mg	Site of infection: NR Other symptoms: NR	Number of patients recruited: NR Number of patients not followed up: NR Samples from 34 patients analysed Number of paired blood samples NR

continued

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; age: NR (85 adults (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Varani *et al.* (2009)[70]	Adults and children; age: NR (85 adults and 15 children) Number 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, AML; 17, ALL; 15, lymphoma; 7, MM; 3, chronic myeloproliferative disorders; 6, solid tumours; 1, autoimmune thrombocytopenia; and 1, haemophagocytic lymphohistiocytosis	100 patients included Number of patients not followed up: NR Samples from 100 patients analysed 130 paired samples
von Lilienfeld-Toal *et al.* (2009)[71]	Adults; median age 60 (IQR 49–66) years 38/70 (54%) male Number immunocompromised: NR Antibiotics prior to blood sample: none Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease: NR	NR	AML, 50 (71%); ALL, 8 (11%); NHD, 4 (6%); MM, 2 (3%); MDS, 2 (3%); aplastic anaemia, 2 (3%); metastatic carcinoma, 2 (3%)	Number of patients recruited: NR Number of patients not followed up: NR 70 patients analysed (119 episodes) 784 paired blood samples

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Westh et al. (2009)[72]	Adults/children/neonates: NR; age: NR	Suspected, severe, shock: NR	NR	Site of infection: NR	Number of patients recruited: NR
	Number male: NR	Severity of disease: NR		Other symptoms: NR	Number of patients not followed up: NR
	Number immunocompromised: NR				Samples from 359 patients analysed
	Limited details provided regarding patients receiving antibiotic treatment at time of blood collection				558 paired samples
	Previous antibiotic exposure: NR				
Berger et al. (2010)[73]	Neonates; age: NR	Suspected, severe, shock: NR	Clinical sepsis suspicion – sepsis not defined	Site of infection: NR	38 patients included
	Number male: NR	Severity of disease: NR		Other symptoms: NR	Number of patients not followed up: NR
	Antibiotics prior to blood sample: NR				Samples from 38 patients analysed
	Previous antibiotic exposure: NR				38 paired samples
Bloos et al. (2010)[74]	Adults; aged 66 years	100% severe sepsis or septic shock	Severe sepsis or septic shock according to ACCP/SCCM consensus criteria[2]	Lung (40%), abdomen (16.9%), bloodstream (9.3%), catheter-related (9.3%)	Number of patients recruited: NR
	68.5% male	Severity of disease SOFA score of 10 for entire sepsis cohort		Mechanical ventilation (81.7%)	Number of patients not followed up: NR
	None immunocompromised				Samples from 142 patients analysed
	95.8% on antibiotics (unclear if prior to blood sampling)				236 paired samples
	Previous antibiotic exposure: NR				

continued

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Lamoth et al. (2010)[75]	Adults; median age 54 (range 17–71) years 53/86 (62%) male Number immunocompromised: NR Antibiotics prior to blood sample 144 (61%) samples drawn under AB therapy Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease: NR	NR	AML, 37 (43%); ALL, 9 (10%); lymphoma, 12 (14%); MM, 22 (26%); other haematological malignancies, 6 (7%); chemotherapy induction/consolidation for acute leukaemia, 45 (52%); autologous stem cell transplant, 37 (43%); other chemotherapy, 4 (5%)	Number of patients recruited: NR Number of patients not followed up: NR 86 patients analysed 237 paired blood samples
Lehmann et al. (2010)[76]	Adults (range 18–84); age 58.37 years 72/108 (67%) male Number 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); 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 comorbidities (some had multiple): neoplasms (18.5%); liver failure (16.7%); NYHA III/IV heart failure (50.9%); chronic renal failure (45.4%); diabetes (19.4%)	Number of patients recruited: NR Number of patients not followed up: NR Samples from 108 patients analysed 453 paired samples

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Maubon et al. (2010)[77]	Adults/children/neonates: NR; age: 56.3 (13.7) years 57/110 (60.9%) male Number immunocompromised: NR (all haemato-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
Regueiro et al. (2010)[78]	Adults, 21–92; aged 64 (range 21–92) years 53/72 (73.6%) male Number 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 scores: 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; neurological, 2; various causes, 6; other cause, 2	72 patients included Number of patients not followed up: NR Samples from 72 patients analysed 106 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Soki et al. (2010)[79]	Adults/children/neonates: NR; age: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 159 patients analysed 162 paired samples
Tsalik et al. (2010)[80]	Adults, 18–97; aged 54.1 (range 18–97) years 168/306 (54.9%) male Number 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, haematological damage, pulmonary damage or renal damage. Sepsis in the presence of hypotension, despite fluid challenge, or a blood lactate concentration of 4 mmol/l 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 Number of patients not followed up: NR Samples from 306 patients analysed 306 paired samples

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Wallet et al. (2010)[81]	Adults; age: NR Number male: NR Number 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 Number of patients not followed up: NR Samples from 72 patients analysed 102 paired samples
Yanagihara et al. (2010)[82]	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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 212 patients analysed 400 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Bravo *et al.* (2011)[83]	Adult; aged 65.5 median (range 23–86) years 33/53 (62%) male Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 53 patients analysed 53 paired samples
Hettwer *et al.* (2011)[84]	Adults; aged 62.3 (± 18.1) years 94 (61%) male Number 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)[85]	Adults and children (range 14–98 years); aged 67 years (median) 607/1093 (56%) male Number 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; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Lucignano et al. (2011)[86]	Neonates and children; age: NR Number male: NR Number 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	811 patients recruited 8 not followed up Samples from 803 patients analysed 1553 paired samples
Obara et al. (2011)[87]	Adults (30–86); aged 61.6 (range 30–86) years 35/54 (64.8%) male Number 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 Number of patients not followed up: NR Samples from 54 patients analysed 78 paired samples
Vrioni et al. (2011)[88]	Adults/children/neonates: NR; age: NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 33 patients analysed 33 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Alvarez *et al.* (2012)[89]	Adults; mean age 64.9 years 55 (54.5%) male Number immunocompromised: NR Antibiotics prior to blood sample: NR Previous antibiotic exposure: NR	All had severe sepsis or septic shock	Dellinger *et al.*[154] and by Levy *et al.*[155]	Emergency abdominal surgery: BC = 9, SF = 11; elective abdominal 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 102 patients analysed Number of paired blood samples: NR
Grif *et al.* (2012)[90]	Adults/children/neonates: NR; age 55.6 years 42/61 (69%) male Number 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 two SIRS criteria – sepsis not defined	Site of infection: NR Other symptoms: NR	61 patients included Number of patients not followed up: NR Samples from 61 patients analysed 71 paired samples

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Guido et al. (2012)[91]	Adults; age 66.1 median (range 23–82) years 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 Number of patients not followed up: NR Samples from 166 patients analysed 166 paired samples
Lodes et al. (2012)[92]	Adults (20–88 years); age 63.1 (14.1) years 74/104 (71.1%) male Number 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	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 Number of patients not followed up: NR Samples from 104 patients analysed 148 paired samples

continued

TABLE 57 Population characteristics *(continued)*

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Mauro *et al.* (2012)[93]	Adults and children; aged 5–68 years 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, ALL; 2, Wilms' tumour; 3, hepatocellular carcinoma; 2, allogeneic stem cell transplantations; 18, NHD; 4, colon cancer under chemotherapy; 2, ovarian cancer; 1, leishmania visceral; and 32, exposure to glucocorticoids for autoimmune disease	79 patients included Number of patients not followed up: NR Samples from 79 patients analysed 79 paired samples
Pasqualini *et al.* (2012)[94]	Adults; aged 73 median (range 20–99) years 215/391 (55%) male 17 (4%) immune deficiency 191/391 (48.8%) had been receiving antibiotic treatment for ≥24 hours 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 Number of patients not followed up: NR Samples from 391 patients analysed 391 paired samples

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Rath et al. (2012)[95]	Adults; aged 52.6 (10.9) (range 27–70) years 72 (64.6%) male Number 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 ischaemia, 4 (4.3%)	170 patients included Number of patients not followed up: NR Samples from 170 patients analysed 225 paired samples
Tschiedel et al. (2012)[96]	Adults and children; median age 6 (range 0–24) years 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, and renal insufficiency with dialysis. 14 samples (13%) were taken from previously healthy patients	75 patients included Number of patients not followed up: NR Samples from 75 patients analysed 110 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Herne *et al.* (2013)[97]	Adults; aged 58 (range 20–81) years 61/144 (42%) male Number immunocompromised: NR (haemato-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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 144 patients analysed 160 paired samples
Kasper and Altiok (2013)[98]	Neonates of very low birthweight; age: NR (however, for blood culture-positive sepsis and blood culture-negative clinical sepsis group, age range 23.3–30.1 weeks) Number male: NR Number immunocompromised: NR [neonates mean (assumed) birthweight 818 ± 242 g] 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 Number of patients not followed up: NR Samples from 46 patients analysed Number of paired blood samples: NR

Author (year)	Adults/children/neonates; age (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Paolucci et al. (2013)[99]	Adults and children; age: NR (23 children, 178 adults) Number male: NR Number immunocompromised: NR (included two cases of autoimmune thrombocytopenia) Antibiotics prior to blood sample: none Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease: NR	NR	Haematological malignancies (105 AML, 23 ALL, 34 lymphoma, 15 MM and 8 chronic myeloproliferative disorders), severe aplastic anaemia (4 patients), solid tumours (9 patients) or other disorders (2 cases of autoimmune thrombocytopenia, 1 case of haemophagocytic lymphohistiocytosis)	Number of patients recruited: NR Number of patients not followed up: NR 201 patients analysed (339 episodes) 437 paired blood samples
Rodrigues et al. (2013)[100]	Adults; age SF: 63 (46–75) years, BC: 66 (39–85) years 31/46 (67%) Male Number immunocompromised: NR Antibiotics prior to blood sample: none Previous antibiotic exposure: none	Septic shock SF: 9/17 (53), BC: 16/29 (55)	NR	Site of infection: NR Other symptoms: NR	Number of patients recruited: NR 46 patients analysed Number of paired blood samples: NR
Avolio et al. (2014)[101]	Adults; age: NR Number 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 SIRS criteria	Site of infection: NR Other symptoms: NR	830 patients included 305 cases did not have a BC 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

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Burdino et al. (2014)[102]	Adults; age NR Number male: NR Number 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 SIRS with suspected bacterial or fungal infections	Site of infection: NR Other symptoms: NR	1024 patients included Number of patients not followed up: NR Samples from 1024 patients analysed 1186 paired samples
Mancini et al. (2014)[103]	Adults; mean age 48.6 years 152/228 (67%) male Number immunocompromised: NR [100% haematological (55.7% with AML)] Antibiotics prior to blood sample: NR Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease: NR	Sepsis was defined according to Dellinger et al.[16]	Site of infection: NR Other symptoms: NR	Number of 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 Number of paired blood samples: NR

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Markota *et al.* (2014)[104]	Adults; aged 59.5 (14.8) years 38/57 (66.7%) male Number 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.*[154]	Site of infection: NR Other symptoms: NR	Number of patients recruited: NR Number of patients not followed up: NR Samples from 57 patients analysed 63 paired samples
Ozkaya-Parlakay *et al.* (2014)[105]	Children; aged 1 month to 17 years, age 2.71 (4.11) years (73.4% < 2 years) 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 69 patients analysed 79 paired samples
Schaub *et al.* (2014)[106]	Adults; aged ≥ 18 years, median 64 years 56/110 (60%) male 15/110 (14%) immunosuppressed (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 patients (17%) and septic shock in five patients (6%)	Sepsis and its severity were defined according to the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference.[155] 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 and throat, 5 (5%); other 3 (3%); systemic, 5 (5%); no focus found, 3 (3%) Diabetes, 26 (24%); renal impairment, 22 (20%); immunosuppression, 15 (14%)	Number of patients recruited: NR Number of patients not followed up: NR Samples from 110 patients analysed 205 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Sitnik et al. (2014)[107]	Adults; aged 49.7 (24.8) years 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[4]	Site of infection: NR Other symptoms: NR	Number of patients recruited: NR Number of patients not followed up: NR Samples from 114 patients analysed 114 paired samples
Barbanti et al. (2015)[108]	Adults/children: NR; age: NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR 491 patients analysed Number of paired blood samples: NR
Calitri et al. (2015)[109]	Children and neonates (8 preterm newborns); median age 6.8 (IQR 2.7–13.1) years 183/289 (63.3%) male Number 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	Suspected, severe, shock: NR Severity of disease: NR	International Paediatric Sepsis Consensus[156]	Site of infection: NR Other symptoms: NR	Number of patients recruited: NR Number of patients not followed up: NR 289 patients analysed (545 episodes) Number of paired blood samples: NR

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Idelevich et al. (2015)[110]	Adults; mean age 52.4 years [SF, 50.4 (14.4) years; BC, 54.4 (15.2) years] 89/150 (59.3%) male [SF, 45/74 (60.8%); BC, 44/76 (57.9%)] Number immunocompromised: NR Antibiotics prior to blood sample 150/150 (100%) Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease: NR	Sepsis was defined according to the SCCM/ACCP consensus conference guidelines of 1992[4]	AML SF = 33 (44.6), BC = 42 (55.3); ALL SF = 8 (10.8), BC = 12 (15.8); MM SF = 12 (16.2), BC = 11 (14.5); NHD SF = 18 (24.3), BC = 6 (7.9); chronic myeloid leukaemia SF = 1 (1.4), BC = 3 (3.9); MDS SF = 1 (1.4), BC = 1 (1.3); others SF = 1 (1.4), BC = 1 (1.3)	Number of patients recruited: NR Number of patients not followed up: NR 150 patients analysed 253 paired blood samples
Tafelski et al. (2015)[112]	Adults (range 47–74 years); age BC + SF: 67 years (median); BC: 59 years (median) BC + SF: 26 (63%) male; BC: 24 (65%) male BC + SF: 6/41 (15%); BC: 6/37 (16%) immunocompromised Antibiotics prior to blood sample: NR Previous antibiotic exposure: NR	Septic shock BC + SF: 20/41 (49%) BC: 25/37 (68%) Median SAPS II on admission: BC + SF 40 (IQR 32–50); BC: 47 (IQR 34–65)	Sepsis defined as suspected or proven infection causing systemic inflammation with at least two of the following: (1) leucocyte count < 4 or > 12/nl; (2) body temperature < 36 °C or fever > 38 °C; (3) tachypnoea > 20 breaths per minute or hyperventilation (PaCO$_2$ < 32 mmHg); (4) tachycardia > 90 beats per minute	Abdominal BC + SF: 15/41 (37%); BC: 8/37 (22%); pulmonary BC + SF: 26/41 (63%); BC: 29/37 (78%) Other symptoms: NR	100 patients included 22 (unable to provide informed consent) not followed up Samples from 78 BC + SF: 41; BC: 37 patients analysed 78 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Warhurst *et al.* (2015)[111]	Adults and children; aged ≥ 16 years, median 58 (44 to 68) years 553/795 (60%) male Number 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.*[155]	Site of infection: NR Other symptoms: NR	795 patients included Number of patients not followed up: NR Samples from 795 (922 episodes) patients analysed Number of paired blood samples: NR
Single index test studies – SepsiTest					
Wellinghausen *et al.* (2009)[48]	173 adults and 14 children <18 years; age: NR Number male: NR Number immunocompromised: none 8 of the 13 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	Site of infection: NR Other symptoms: NR	Number of patients recruited: NR Number of patients not followed up: NR Samples from 187 patients analysed 342 paired samples

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Loonen et al. (2014)[114]	Adults; positive blood culture aged 68.9 (17.3) years; negative blood culture aged 60.4 (18.0) years Positive blood culture, 17/125 (13.6%) male, negative blood culture, 57/125 (45.6%) male Number 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 Number of paired blood samples: NR
Nieman et al. (2015)[113]	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed
Single index test studies: IRIDICA					
Bacconi et al. (2014)[49]	Adults; aged ≥ 18 years Number male: NR Number 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 Number of patients not followed up: NR Samples from 331 patients analysed 331 paired samples

TABLE 57 Population characteristics (*continued*)

Author (year)	Adults/children/neonates; mean (SD) age, *n/N* (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Delco-Volante *et al.* (2015)[115]	Neonates (<28 days old); age: NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Number of patients analysed: NR 81 paired samples
Vincent *et al.* (2015)[116]	Adults; aged 61 (17.9) years 64.6% male Immunosuppressed, 90/543 (16.6%) Within 30 days prior to hospitalisation, 12.7%; initiated during hospitalisation, 62.7%; initiated at or after enrolment: 22.1% Previous antibiotic exposure: NR	Suspected, severe, shock: NR Severity of disease entire cohort: mean SOFA score at baseline: 7.6 (4.2) indicating 15–20% risk in mortality in ICU	NR	Site of infection: NR Other symptoms: NR	543 patients included 14 did not have matching PCR/ESI-MS or standard-of-care microbiology results not followed up Samples from 529 patients analysed Number of paired blood samples: NR
Metzgar (2015)[117]	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed

Two index test studies: SeptiFast and SepsiTest

Author (year)	Adults/children/neonates; mean (SD) age, n/N (%) males; number immunocompromised; number 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	Number of patients recruited/included, not followed up, analysed; number of paired blood samples
Leitner et al. (2013)[118]	Adults/children/neonates: NR; age: NR Number male: NR Number 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	Number of patients recruited: NR Number of patients not followed up: NR Samples from 57 patients analysed 75 paired samples
Schreiber and Nierhaus (2013)[119]	Adults; age median 64 (IQR 51–70) years 10/50 (20%) male Number 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 33–49)	Diagnosis of sepsis according to the sepsis criteria of the German Sepsis Competence Network[153]	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)	Number of patients recruited: NR Number of patients not followed up: NR Samples from 50 patients analysed Number of paired blood samples: NR

ACCP, American College of Chest Physicians; AIDS, acquired immunodeficiency syndrome; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ARDS, acute respiratory distress syndrome; ATS, American Thoracic Society; BC, blood culture; Biph. AL, biphenotypic acute leukaemia; CLL, chronic lymphatic leukaemia; CNS, central nervous system; CV, cardiovascular; ED, emergency department; ESI, electrospray ionisation; ESICM, European Society of Intensive Care Medicine; HD, Hodgkin's lymphoma; HIV, human immunodeficiency virus; IQR, interquartile range; MDS, myelodysplastic syndrome; MM, multiple myeloma; MODS, multiple organ dysfunction syndrome; NASH, non-alcoholic steatohepatitis; NHD, non-Hodgkin's lymphoma; NR, not reported; NYHA, New York Heart Association; PaCO$_2$, partial pressure of carbon dioxide; SCCM, Society of Critical Care Medicine; SF, SeptiFast; SIS, Surgical Thoracic Society.

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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Single index test studies: SeptiFast								
Raglio et al. (2006)[44]	LightCycler SeptiFast Test (MGRADE NR) 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: NR Sampling method: NR Culture method: NR	NR	NR	Sample	Included
Bingold et al. (2007)[59]	LightCycler SeptiFast Test (MGRADE NR) 3 ml of kEDTA 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
Klemm et al. (2007)[60]	LightCycler SeptiFast Test (MGRADE NR) 2 × 3.5 ml of 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 × 10 ml of blood BACTEC BD (Becton, Dickinson and Company, Franklin Lakes, NJ, USA)	NR	NR	Patients	NR (four samples contaminated with dermal Streptococci deliberately not reported by SeptiFast interpretive software)
Lodes et al. (2008)[61]	LightCycler SeptiFast Test (MGRADE NR) 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: NR 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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Louie et al. (2008)[46]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: NR Volume: 3 ml of 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) Culture method: NR	NR	NR	Patients	Excluded
Mancini et al. (2008)[45]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: at least 1.5 ml of 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 20 ml of blood (average of 25 ml inoculated into aerobic and anaerobic bottles) BacT/ALERT 3D automated blood culture system (bioMérieux, Marcy-l'Etoile, France), with monitoring of carbon dioxide production within each bottle every 10 minutes 24 hours 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 (bioMérieux, Marcy-l'Etoile, France)	NR	7 days per week (from 8 a.m. to 7 p.m.)	Samples	Included

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Vince et al. (2008)[62]	LightCycler SeptiFast Test (MGRADE NR) 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: NR Sampling method: NR Culture method: NR	NR	NR	Samples	NR
Dark et al. (2009)[63]	LightCycler SeptiFast Test (MGRADE NR) Sample: NR Site: NR Volume: 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 (three detected in blood culture but not in SeptiFast, assigned as true negatives)
Dierkes et al. (2009)[64]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: NR Volume: 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 Sample: whole blood Site: NR Volume: 2 × 10-ml bottles BACTEC 9240. Both aerobic and anaerobic blood culture bottles were inoculated directly with 10 ml of 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	7 days per week (Monday to Friday from 8 a.m. to 7 p.m.; Saturday, Sunday and holidays from 9 a.m. to 4 p.m.)	Samples	Included

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Gimeno et al. (2009)[65]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: venous or catheter Volume: 3 ml 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)[66]	LightCycler SeptiFast Test (MGRADE NR) 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 blood culture 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)[67]	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: NR Sampling method: NR Culture method: NR	NR	NR	Samples	Included

continued

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Palomares et al. (2009)[68]	LightCycler SeptiFast Test (MGRADE NR) 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: NR Sampling method: NR Culture method: NR	NR	NR	Sample	Included
Paolucci et al. (2009)[69]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: peripheral venous Volume: 1.5 ml When samples drawn: NR	Yes	NR	Blood culture plus clinical. Bloodstream infection was confirmed by the presence of clinical signs of infection or additional microbiological data Sample: whole blood Site: peripheral venous Volume: 1.0 ml of blood Blood cultures were performed according to the 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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Varani et al. (2009)[70]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: adults, peripheral veins; children, central venous catheter Volume: K-EDTA 3 ml was sampled and processed for patients who weighed 45 kg and 1.5 ml for those who weighed <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 CLSI	NR	NR	Febrile episodes	Included
von Lilienfeld-Toal et al. (2009)[71]	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 [1 ml (adults)]	NR	Blood culture was performed using the BACTEC system	NR	NR	Pathogen	NR

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Westh *et al.* (2009)[72]	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 (5 ml drawn) 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 micro-organisms from a suspected infectious focus within 48 hours of the episode was used to resolve discrepancies in the results Sample: whole blood Site: venous Volume: 8–10 ml of blood for each aerobic and anaerobic bottle for each system	NR	NR	Pathogen	Excluded
Berger *et al.* (2010)[73]	LightCycler SeptiFast Test (MGRADE NR) Modified DNA extraction for very low birthweight 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 Volume: 0.1 ml of blood Sampling method: NR	NR	NR	Patients	Included

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Bloos et al. (2010)[74]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: venous Volume: 10 ml, 3 ml from 10 ml EDTA treated Reference standard and index tests performed on blood samples drawn at the same time	No [3 ml (adults)]	Same day as severe sepsis suspected	Blood culture and clinical/laboratory confirmation Whole blood (20 ml) for conventional cultures Pair of blood cultures incubated at 37 °C and monitored for 8 days. Isolated micro-organisms 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
Lamoth et al. (2010)[75]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: venous Volume: 1 ml Reference standard and index tests performed on blood samples drawn at the same time	No [3 ml (adults)]	For SeptiFast assays, DNA was extracted from the EDTA whole-blood tubes within 48–72 hours after sampling	Blood culture was performed using the BACTEC system	NR	NR	Episodes	NR

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Lehmann et al. (2010)[76]	SeptiFast Prep Kit and the LightCycler (MGRADE NR) Sample: whole blood Site: NR Volume: used 1 ml 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 (sensitivity 0.83 and specificity 0.93) Sample: whole blood Site: NR Volume: 20 µl for pair of aerobic and anaerobic blood culture bottles Blood culture was performed using the BACTEC system. All blood cultures 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 bloodstream infection was defined as a positive blood culture result, obtained and analysed as set forth by the current DGHM procedures. Whether micro-organisms identified by PCR represented true infection or contamination was evaluated retrospectively by taking into account the identity of the micro-organism detected and by comparing PCR results with corresponding blood culture findings	NR (however laboratories were not 24 hours a day, 7 days per week)	Samples	Included

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Maubon et al. (2010)[77]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: 1.5 ml EDTA When samples drawn: NR	Yes	NR	Blood culture plus clinical: leucocyte 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: NR Sampling method: NR Culture method: NR	NR	NR	Patients	Unclear
Regueiro et al. (2010)[78]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: venous or arterial draw Volume: 1.5 ml 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: 10 ml 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

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Soki *et al.* (2010)[79]	SeptiFast Test (MGRADE NR) Sample: whole blood Site: NR Volume: NR When samples drawn: NR	Unclear	NR	Blood culture: NR if with clinical confirmation or not Sample: whole blood Site: NR Volume: NR Blood culture was performed using the BACTEC system. Blood culture sets (one aerobic and one anaerobic bottle) were used	NR	NR	Sample	NR
Tsalik *et al.* (2010)[80]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: 1.5 ml When samples drawn: NR	Yes	NR	Blood culture and clinical confirmation 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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Wallet et al. (2010)[81]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: venepuncture, site NR Volume: EDTA 5 ml; volume for DNA preparation was 1.5 ml 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: 10 ml Blood culture was performed using the BacT/ALERT system plus BACTEC	NR	NR	Pathogens	Included
Yanagihara et al. (2010)[82]	SeptiFast-Lys and Prep MGRADE kits Sample: whole blood Site: NR Volume: used 1.5 ml for assay (10 ml drawn) 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 in which results were positive were sent to one commercial laboratory to confirm valid identification of micro-organisms	NR	NR	Sample	Included

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Bravo *et al.* (2011)[83]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: venous Volume: 1.5 ml 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 Volume: paired 10-ml bottles of blood 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 minutes. Direct smear examination (Gram staining) was performed from positive blood cultures as soon as detected, obtained at 30-minute intervals	The significance of either the isolation of a potentially contaminating micro-organism 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
Hettwer *et al.* (2011)[84]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: venous Volume: 10 ml EDTA Reference standard and index text performed on blood samples drawn at the same time	Unclear	While 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 Sample: whole blood Volume: 2 × 10-ml bottles of blood BacT/ALERT analysed at the 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 standardised procedures	NR	NR	Patients	Included

Author (year)	Name of test, sampling method, interval method, between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Josefson *et al.* (2011)[85]	SeptiFast Lys Kit MGRADE and the MagNA Lyser Sample: whole blood Site: venous (for all samples) Volume: 1.5 ml 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 hours 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 minutes 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 micro-organism was isolated in the blood culture of the same blood culture/PCR set	NR	Patients	Included

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Lucignano *et al.* (2011)[86]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: peripheral or central venous line Volume: ≥ 1.5 ml 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 laboratory variables (leucocyte count, C-reactive protein, microbiological evidence of infection focus) Sample: whole blood Site: peripheral or central venous line Volume: 0.5–10 ml of blood 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 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 (bioMérieux, Durham, NC, USA) or Phoenix (BD Diagnostics) system]	The condition of sepsis was defined when SIRS was in the presence, or a result, of suspected or proven infection, ascertained by the microbiology routine team, which addressed the final interpretation of the results (contaminants vs. pathogens) on the basis of type of microbe, 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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Obara et al. (2011)[87]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: NR Volume: 1.5 ml 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 confirmation or not Sample: whole blood Site: NR Volume: 5 ml of blood Blood culture was performed using the BACTEC system. After cultivation, the strain was identified by WalkAway 96 SI system and auto scan-4 (Dade Behring GmbH, Germany)	NR	NR	Samples	Included
Vrioni et al. (2011)[88]	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: NR Sampling method: NR Culture method: NR	NR	NR	Patients	NR
Alvarez et al. (2012)[89]	LightCycler SeptiFast Test (MGRADE NR) 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, intravenous catheters and other sources. No further detail reported	NR	NR	No diagnostic data reported	No diagnostic data reported

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Grif et al. (2012)[90]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: venous Volume: 1.5 ml processed from 5 ml 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 Sample: whole blood Site: venous Volume: 20 ml BacT/ALERT 3D, two bottles incubated for maximum 5 days at 37 °C. From all bottles signalled as positive, micro-organisms 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
Guido et al. (2012)[91]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: venous Volume: 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 Sample: whole blood Site: venous Volume: 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	NR (but positive blood culture bottles from automated blood culture system removed between 8 a.m. and 7 p.m. for further analysis)	Samples	Included

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Lodes et al. (2012)[92]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: NR Volume: 1 ml EDTA for assay (a pair of aerobic/anaerobic blood culture bottles – 9 ml 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 plus clinical diagnosis (text suggests clinical diagnosis) Sample: whole blood Site: NR Volume: NR Blood culture was performed using the BACTEC system	NR	NR	Samples	Included
Mauro et al. (2012)[93]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: 1.5 ml 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 – one 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 hours, and identification was carried out with a VITEK 2 system	NR	NR	Samples	Included

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Pasqualini et al. (2012)[94]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: 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 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	Micro-organisms 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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Rath et al. (2012)[95]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: venepuncture or central venous catheter Volume: 3 ml 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: 8–10 ml of blood per bottle Blood culture was performed using the BACTEC system	NR	NR	Samples	Included
Tschiedel et al. (2012)[96]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: 1.5 ml 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, laboratories open until 11 p.m. Monday to Friday)	Samples	Included

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Herne *et al.* (2013)[97]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: venous Volume: 1.5 ml from 3 ml K2EDTA All blood culture/SeptiFast samples collected within 12 hours of each other	Yes	Perform SeptiFast from 8 a.m. to 4 p.m. only, 7 days of the week	Blood culture alone and blood culture with clinical and other microbiological cultures Sample: whole blood Site: venous Volume: > 2 × 10 ml bottles BacT/ALERT 3D. At least two sets of 10-ml paired bottles 0.5–1 hour 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 the event of positive blood cultures, micro-organisms 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 4. susceptibility testing by disc diffusion test and/or gradient method for MIC detection accordingly to the identified pathogen and laboratory protocol	Defined all cases in which both methods 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	7 days per week (SeptiFast assay performed from 8 a.m. to 4 p.m. only)	Samples	Included

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Kasper and Altiok (2013)[98]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: 0.1–0.7 ml (maximum) blood sample into 0.8 ml K3EDTA vacutainers Reference standard and index tests performed on blood samples drawn at the same time	No (0.1–0.7 ml, neonates)	Samples analysed immediately or stored at –20 °C and processed next day	Blood culture plus clinical diagnosis Sample: whole blood Site: NR Volume: 0.5–1.0 ml of blood Blood culture was performed using the BacT/ALERT system. Only aerobic paediatric bottle was inoculated and incubated for 7 days (because of limited blood volumes) by standard microbiological procedures	NR	NR	Patients	Included
Paolucci et al. (2013)[99]	LightCycler SeptiFast Test (MGRADE NR) Sample: NR Site: NR Volume: NR Reference standard and index tests performed on blood samples drawn at the same time	No (3 ml, adults)	Blood samples intended for PCR analyses arriving to the laboratory after 12 p.m. were stored at 4 °C until the next PCR session was programmed	Blood culture was performed using the BacT/ALERT system	NR	Blood cultures were accepted for automated incubation from 8 a.m. to 7 p.m. (Monday to Saturday). PCR was performed once per day from Monday to Friday (samples were received by 12 p.m.). Blood samples intended for PCR analyses arriving to the laboratory after 12 p.m. were stored at 4 °C until the next PCR session was programmed (time limit for storage was 72 hours, as suggested by the manufacturer)	Episodes	Excluded

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Rodrigues *et al.* (2013)[100]	LightCycler SeptiFast Test (MGRADE NR) 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 was performed using the BacT/ALERT system	NR	NR	Incomplete diagnostic data reported	Incomplete diagnostic data reported
Avolio *et al.* (2014)[101]	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 hours	Blood culture and clinical/ laboratory confirmation A blood culture bottle of 20 ml of blood either by venepuncture or from an intravenous access device BacT/ALERT 3D automated system (bioMérieux). All instances in which a maximum of three sets (six bottles) of blood cultures for patient, obtained during a 24-hour period and arrived simultaneously at laboratory was included. When aerobic/ anaerobic bottles gave a positive signal, Gram staining was carried out	Micro-organisms 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	7 days per week (Monday to Friday from 7:30 a.m. to 5 p.m.; Saturday and Sunday from 7:30 a.m. to 12:30 p.m.)	Pathogen	Excluded

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Burdino et al. (2014)[102]	LightCycler SeptiFast Test MGRADE Sample: whole blood Sire: venous Volume: 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 vs. irrelevant and/or contaminants for both blood culture and SeptiFast Sample: whole blood Site: venous Volume: 8–10 ml of blood bioMérieux 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
Mancini et al. (2014)[103]	LightCycler SeptiFast Test (MGRADE NR) 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 organised assuring two daily sessions from Monday to Friday	Incomplete diagnostic data reported	Incomplete diagnostic data reported

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Markota *et al.* (2014)[104]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: peripheral veins Volume: NR but EDTA 5 ml drawn Reference standard and index tests performed on blood samples drawn at the same time	Unclear	On same day if sampled before 6 p.m., next day if sampled after 6 p.m.	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 8 a.m. to 4 p.m. on weekdays and from 8 a.m. to 1 p.m. on Saturdays	Sample	NR
Ozkaya-Parlakay *et al.* (2014)[105]	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 −20 °C	Blood culture and clinical/laboratory confirmation Sample: whole blood Site: venepunctures, site NR Volume: NR 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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Schaub et al. (2014)[106]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: venepunctures, site NR Volume: NR, but collected in 2.7-ml EDTA 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 hours a day, 7 days a week and using the reported turnaround time of the assay of approximately 6 hours	Blood culture and clinical/laboratory confirmation Sample: whole blood Site: venepunctures, site NR Volume: 25 ml 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, for example conventional microbiological methods such as 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 8 a.m. to 5 p.m.)	Patients	Included
Sitnik et al. (2014)[107]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: NR Volume: 5 ml collected from each patient in sterile EDTA tube (mechanical lysis on 3 ml) 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 plated 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 2 system and API 32 °C for yeast	NR	NR	Sample	Included

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Barbanti *et al.* (2015)[108]	LightCycler SeptiFast Test (MGRADE NR) 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 was performed using the BacT/ALERT system	NR	NR	Samples	Included
Calitri *et al.* (2015)[109]	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 a few hours of collection for the SeptiFast test; blood culture collected on same day or if unavailable the nearest blood culture performed was recorded (± 48 hours maximum from SeptiFast collection)	Blood culture was performed using the BacT/ALERT system. Each blood culture consisted of one bottle aerobic or anaerobic cultures, each inoculated with minimum blood required according to paediatric age group	NR	NR	Episodes	Included

Author (year)	Name of test, sampling method, interval method between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Idelevich *et al.* (2015)[110]	LightCycler SeptiFast Test MGRADE Sample: whole blood Site: peripheral veins in adults central venous catheter in children Volume: 3 ml ≤45 kg, 1.5 ml > 45 kg 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 p.m. were stored at 4 °C and processed on the next day. The median time to the arrival of blood culture samples at the microbiological laboratory was 13.3 hours (12.9 hours in the study group and 13.6 hours in the control group; *p* = 0.14). It took 14.8 hours 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	Blood culture was performed using the BACTEC system. The blood culture was incubated for at maximum 7 days	NR	Blood culture Monday to Friday 7:30 a.m. to 6:00 p.m, Saturday 7:30 a.m. to 1:30 p.m.; PCR Monday to Friday 7:30 a.m. to 6:00 p.m.	Pathogen	Included

TABLE 58 Characteristics of the index and reference tests *(continued)*

Author (year)	Name of test, sampling method, interval method between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Tafelski *et al.* (2015)[112]	LightCycler SeptiFast Test (MGRADE NR) Sample: whole blood Site: venous or arterial site Volume: 1.5 ml 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 –80 °C for later analysis	Blood culture plus MALDI-TOF MS with clinical adjudication Sample: whole blood Site: peripheral venous blood Volume: 10 ml 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 (6 a.m. to 6 p.m.)	Samples	NR

continued

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Warhurst et al. (2015)[111]	SeptiFast Test (MGRADE NR)	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	NR	NR	Pathogens	Excluded
	Sample: whole blood							
	Site: Two separate sites (including one peripheral site)			Sample: whole blood				
				Site: two separate site (including one peripheral site)				
	Volume: used 1.5 ml for assay (20 ml drawn)			Volume: two blood samples of 20 ml from two separate sites				
	Reference standard and index tests performed on blood samples drawn at the same time			Blood culture was performed using BacT/ALERT system. Blood cultures entered the standard clinical pathway				

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory 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. Blood cultures 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: 20 ml for adults, 3–5 ml for children

Blood culture was performed using the BACTEC system in adults and BACTEC PED system in children | Probable to true bacteraemia was assigned if:

1. 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
2. the species detected was a typical causative pathogen of the clinical scenario and no other causative pathogen was detected | NR | Samples | Included |
| Loonen et al. (2014)[114] | SepsiTest

Sample: remnant whole blood 1 ml

When samples drawn: NR | Yes | NR | Blood culture plus MALDI-TOF MS and clinical/laboratory confirmation

Remnant whole blood 1 ml

Blood culture was performed using the BacT/ALERT system. Two pairs of aerobic and anaerobic bottles were obtained and incubated for at least 5 days and a maximum of 7 days | NR | NR | Samples | Included |
| Nieman et al. (2015)[113] | Confidential information has been removed | Confidential information has been removed | Confidential information has been removed | Confidential information has been removed | Confidential information has been removed | Confidential information has been removed | Confidential information has been removed | Confidential information has been removed |

Single index test studies – IRIDICA

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Bacconi et al. (2014)[49]	IRIDICA–PLEX-ID Hybrid Sample: whole blood Site: venepuncture, arm Volume: 5–15 ml Reference standard and index tests performed on blood samples drawn within 30 minutes	Yes	Kept at 4 °C within 30-minute 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	NR	Samples	NR
Delco-Volante et al. 2015[115]	IRIDICA Sample: whole blood Site: venepuncture Volume: 0.5 ml 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: NR Sampling method: NR Culture method: NR	NR	NR	Samples	Included

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Vincent *et al.* (2015)[116]	IRIDICA–PLEX-ID Hybrid Sample: whole blood Site: NR Volume: minimum 5 ml Reference standard and index tests performed on blood samples drawn at the same time	Yes	Specimens were cooled to 4 °C within 30 minutes of collection and maintained at 4 °C or frozen at –20 °C until analysis	Blood culture and clinical/laboratory confirmation Sample: whole blood Site: NR Volume: NR Sampling method: NR Culture method: NR	The simple kappa statistic was used to estimate the agreement between culture and PCR/ESI-MS, but it under-represents this agreement because of the requirement that the PCR/ESI-MS assay and blood culture results matched in terms of organism identity in order to be considered a true positive in the contingency table	NR	Samples	Excluded
Metzgar *et al.* (2015)[117]	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed	Confidential information has been removed

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	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Leitner et al. (2013)[118]	SeptiFast Lys Kit and the SeptiFast Prep Kit MGRADE: NR 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 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 a maximum 7 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

TABLE 58 Characteristics of the index and reference tests (*continued*)

Author (year)	Name of test, sampling method, interval between index test and reference standard	CE-approved blood volume	Laboratory test performed on same day as sampling method	Name of reference standard, sampling method, culture methods	Definition of true positive: index test/reference test	Laboratory working times for analysis	Unit of analysis (metric)	Contaminants included?
Schreiber and Nierhaus (2013)[119]	LightCycler SeptiFast Test MGRADE: NR 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 hours, in accordance with the manufacturers' 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 with the final clinical diagnosis and categorised as true positive or false positive and true negative or false negative	NR	Patients	Included

API, Analytical Profile Index; CLSI, Clinical and Laboratory Standards Institute; CoNS, coagulase-negative staphylococci; DGHM, German Society of Medical Microbiology and Hygiene; ESI-MS, electrospray ionisation–mass spectrometry; K2EDTA, di-potassium ethylenediaminetetraacetic acid tubes; K3EDTA, tri-potassium ethylenediaminetetraacetic acid tubes; MIC, minimum inhibitory concentration; MRT, multiplex real time; NA, nucleic acid; NR, not reported; TTP, time to positivity.

Appendix 5 Diagnostic test accuracy, additional information

TABLE 59 Deviance information criterion for SeptiFast compared with blood culture. Standard model (without covariate adjustment) and metaregression models (with covariates indicating subgroups)

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

DIC, deviance information criterion.
a Note that lower values of DIC are favourable, suggesting a more parsimonious model.

TABLE 60 Coefficient estimates for metaregression 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 to 0.78)
Specificity	−0.58 (−1.24 to 0.10)

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

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

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

Search strategy

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

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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 1 assumes 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

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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.

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- 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 with both blood culture and 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
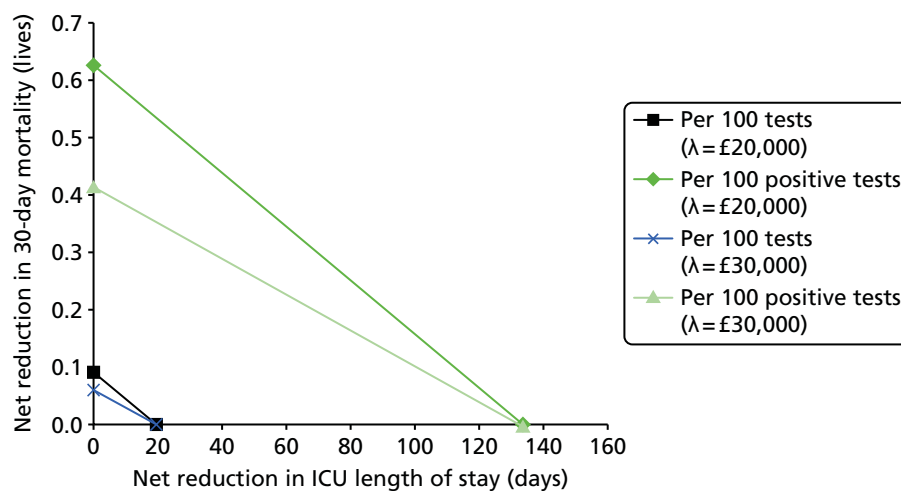
FIGURE 24 Threshold analyses for SeptiFast vs. blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 2.4 samples need to be analysed per day.
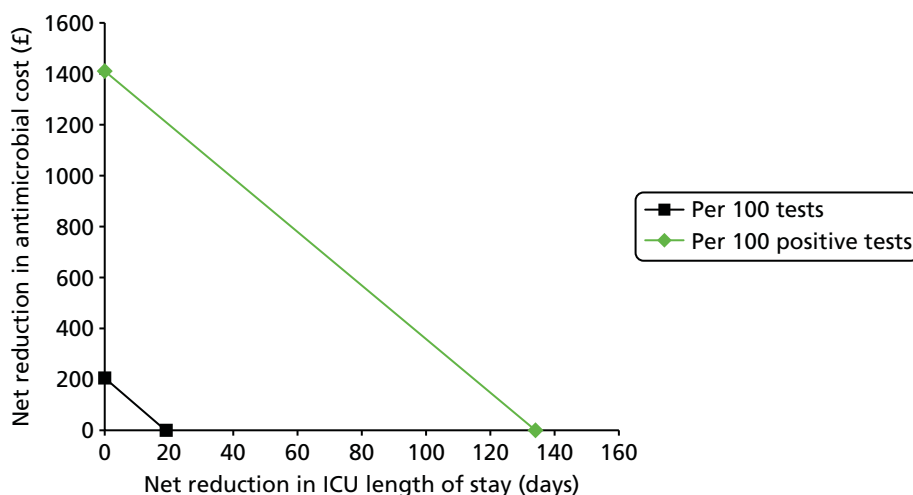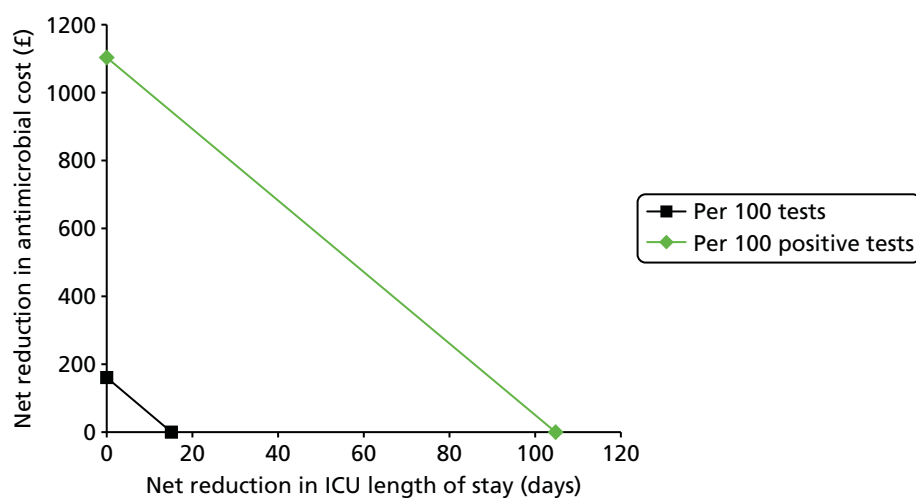

FIGURE 25 Threshold analyses for SeptiFast vs. blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 2.4 samples need to be analysed per day.

Threshold analyses for SeptiFast versus matrix-absorbed laser desorption/ ionisation time-of-flight mass spectrometry
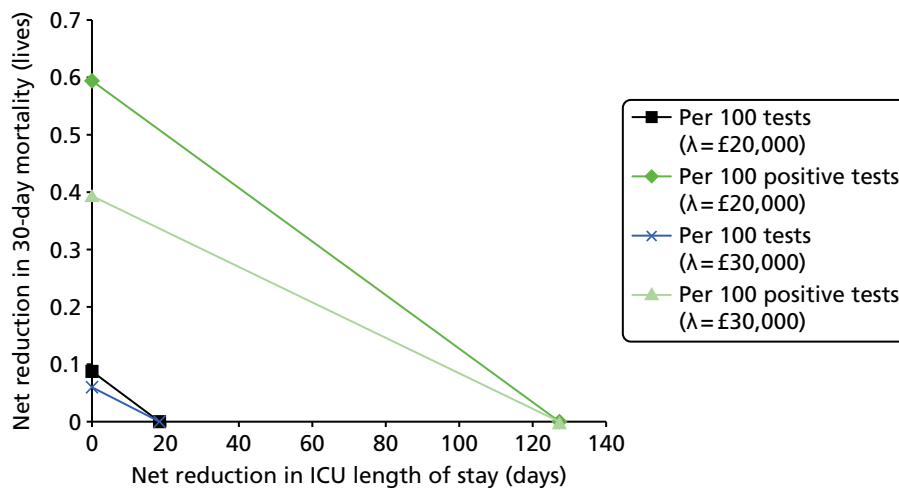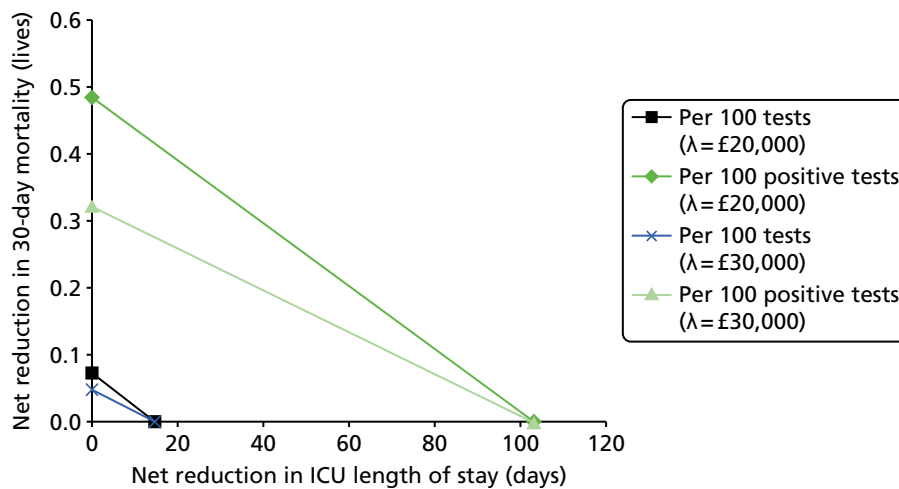

FIGURE 26 Threshold analyses for SeptiFast vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 2.4 samples need to be analysed per day.
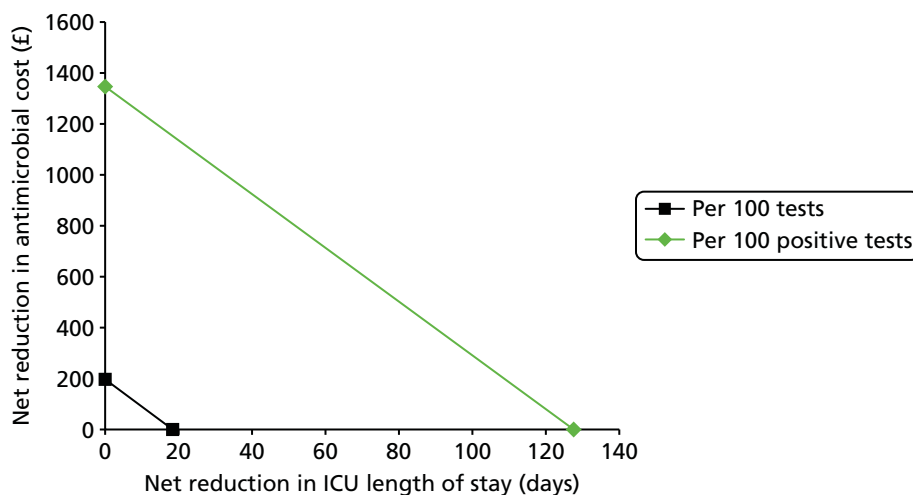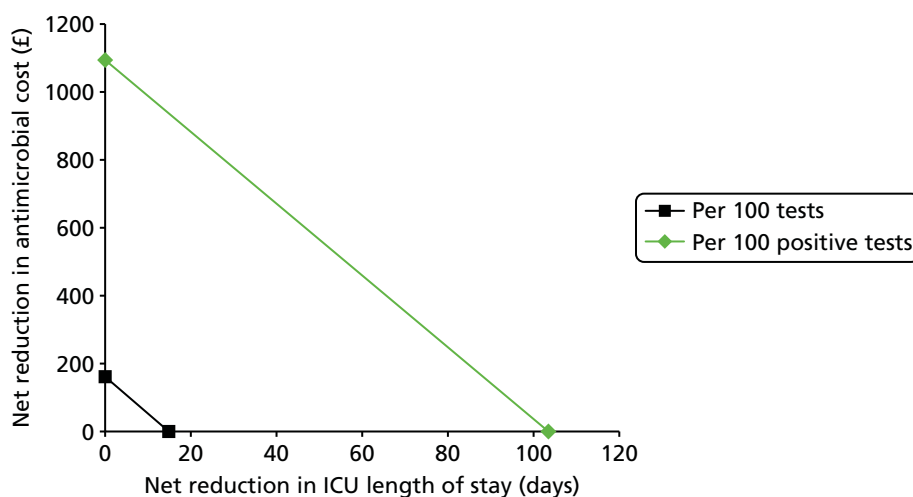

FIGURE 27 Threshold analyses for SeptiFast vs. MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 2.4 samples need to be analysed per day.

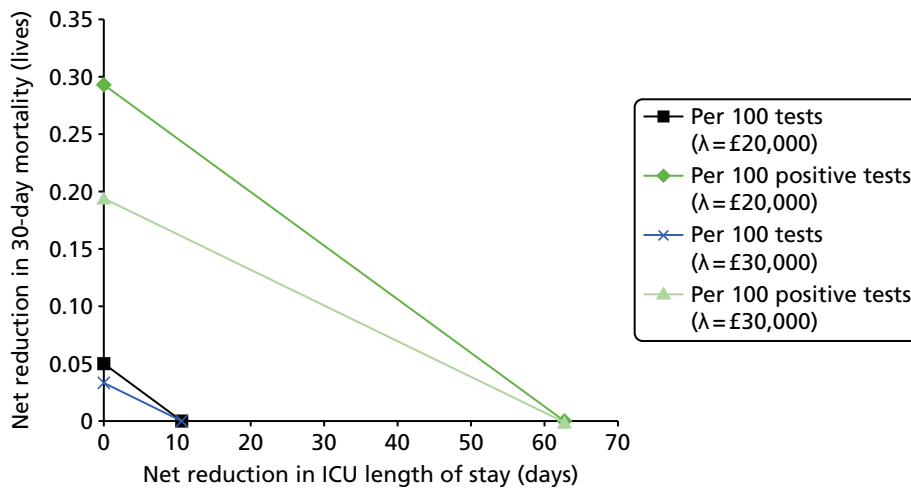### Threshold analyses for SepsiTest versus blood culture
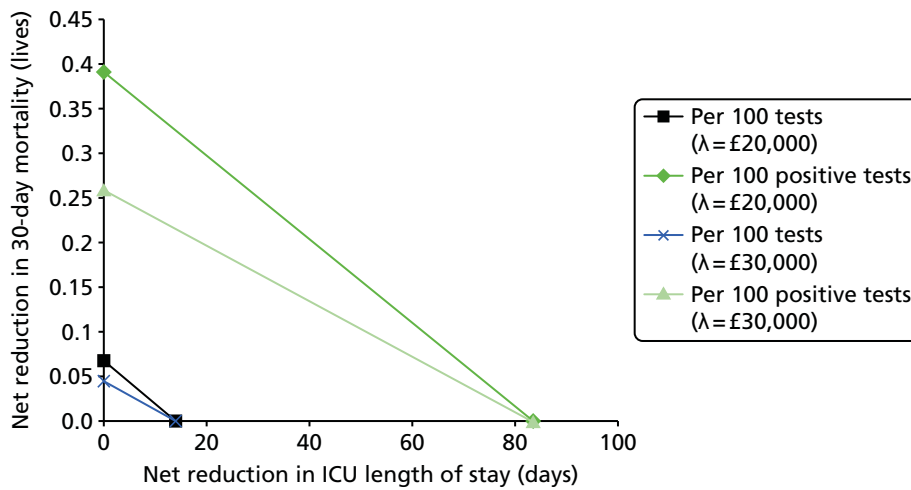
FIGURE 28 Threshold analyses for SepsiTest vs. blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that machinery related to SepsiTest needs to be purchased and 2.4 samples need to be analysed per day.
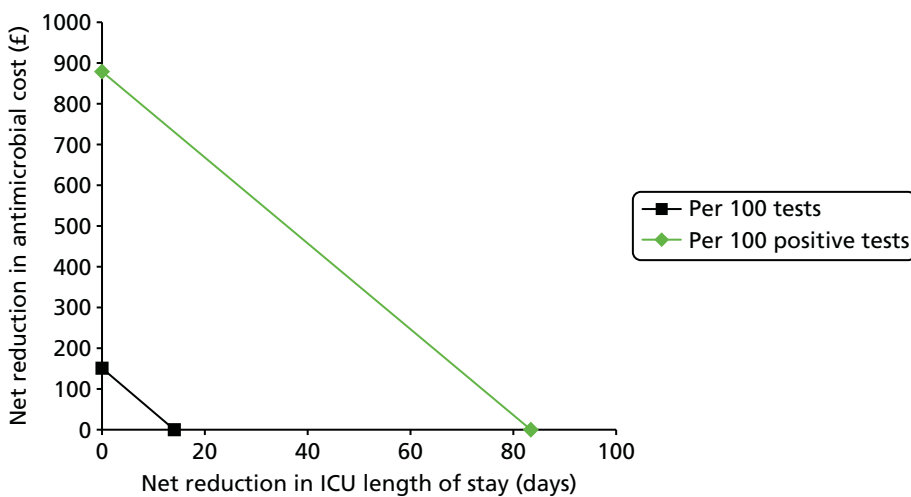
FIGURE 29 Threshold analyses for SepsiTest vs. blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that machinery related to SepsiTest needs to be purchased and 2.4 samples need to be analysed per day.
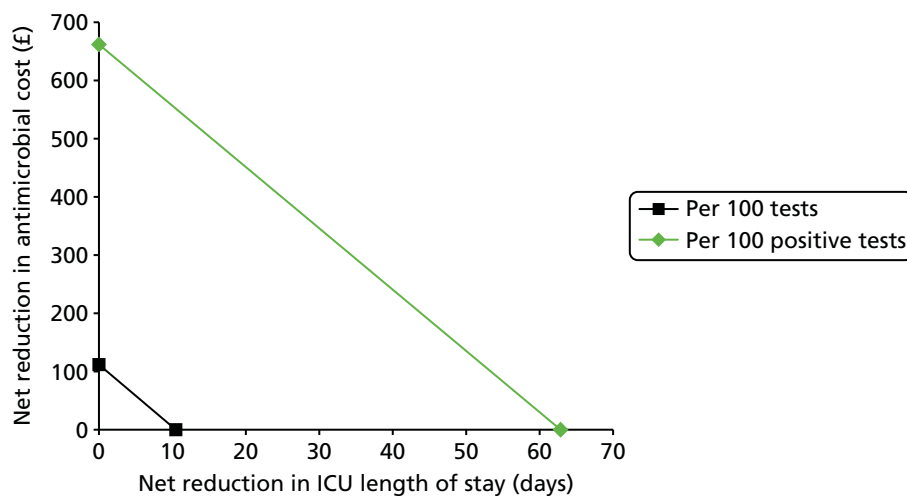
Threshold analyses for SepsiTest versus matrix-absorbed laser desorption/ ionisation time-of-flight mass spectrometry
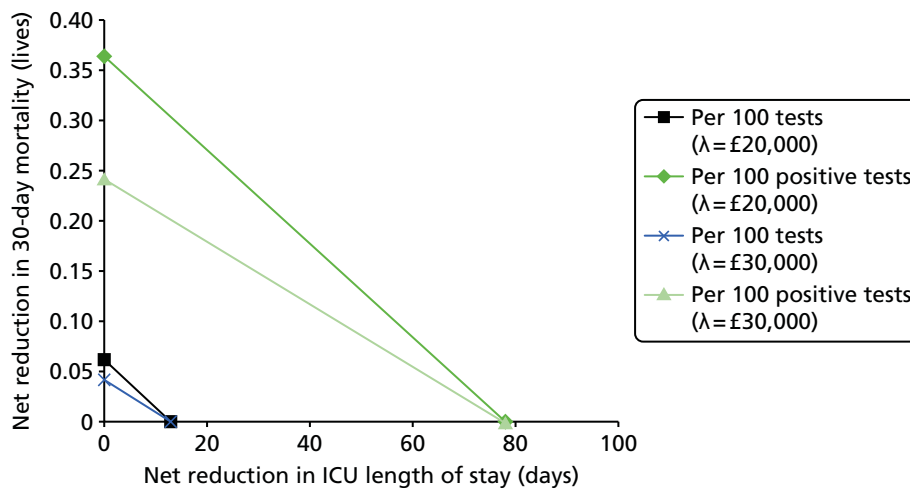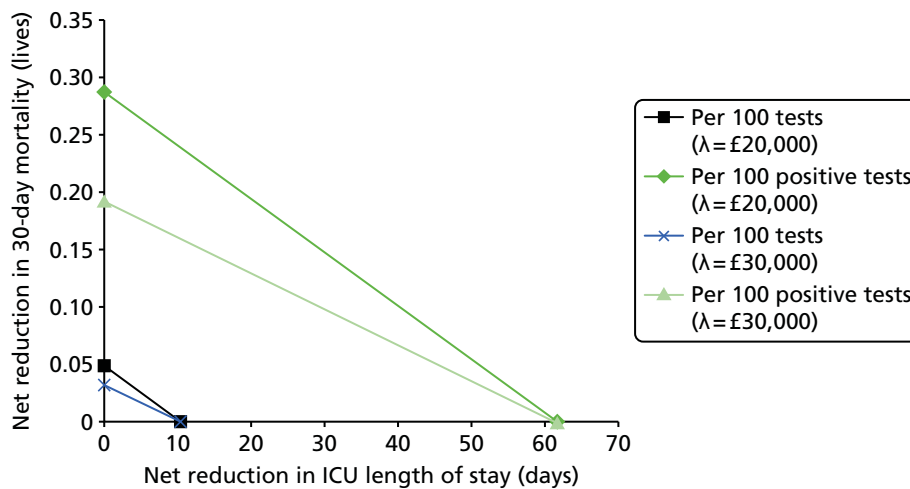

FIGURE 30 Threshold analyses for SepsiTest vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that machinery related to SepsiTest needs to be purchased and 2.4 samples need to be analysed per day.
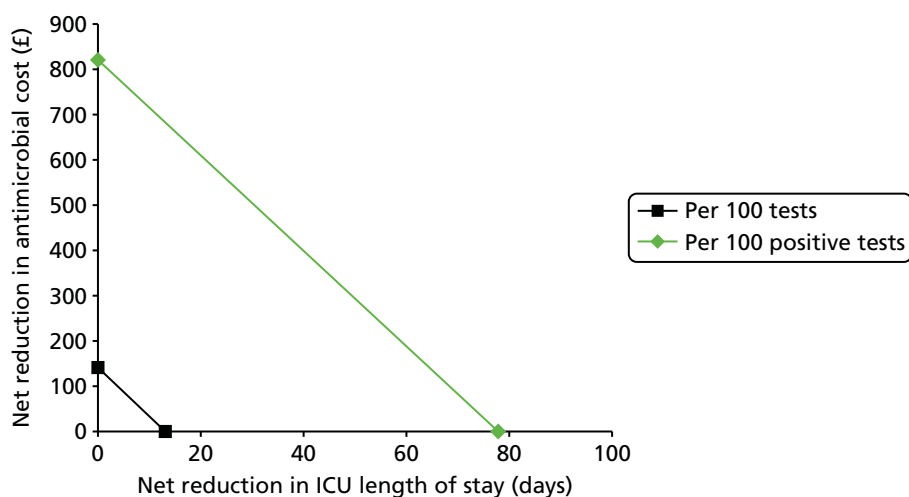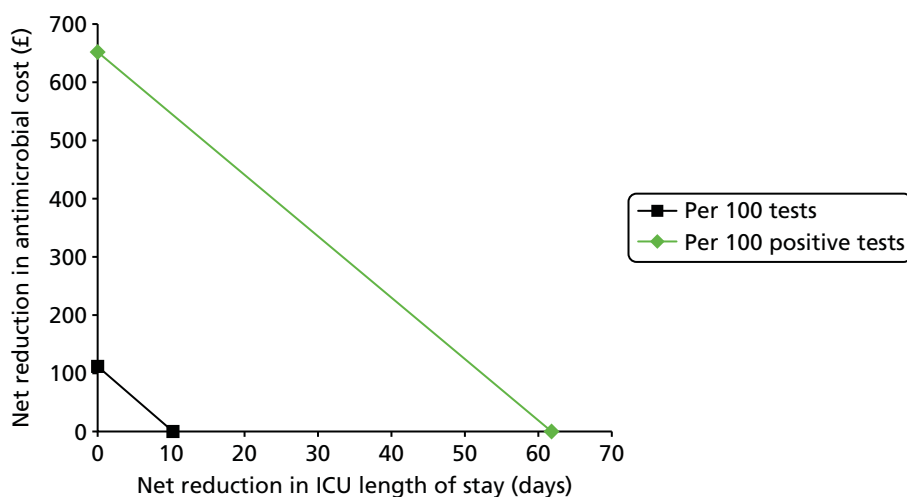

FIGURE 31 Threshold analyses for SepsiTest vs. MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that machinery related to SepsiTest needs to be purchased and 2.4 samples need to be analysed per day.

Threshold analyses for IRIDICA versus blood culture
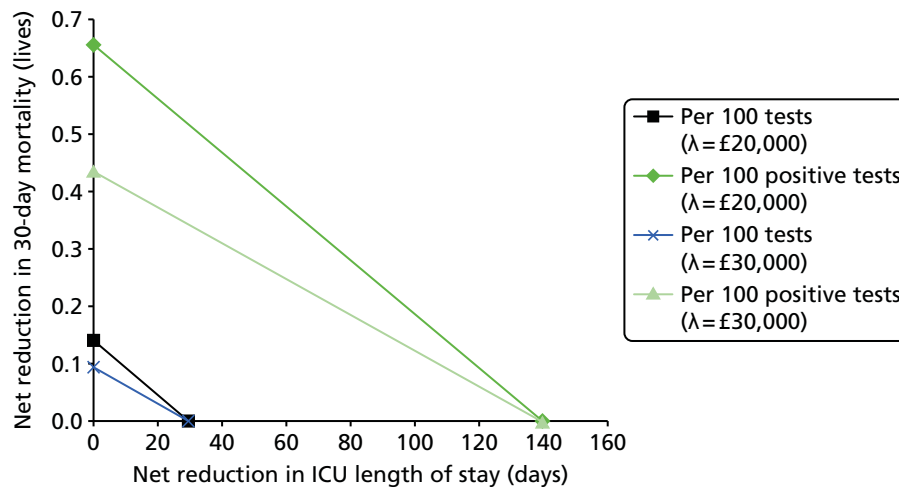
FIGURE 32 Threshold analyses for IRIDICA vs. blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 2.4 samples need to be analysed per day.
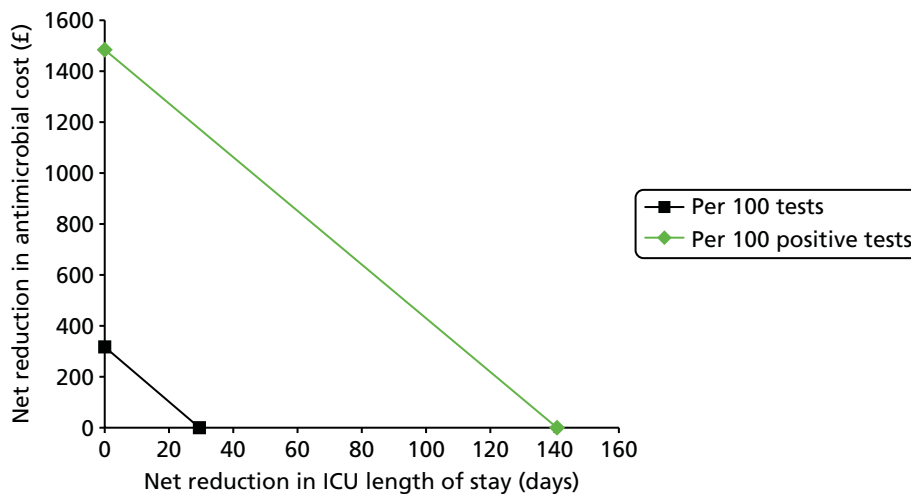
FIGURE 33 Threshold analyses for IRIDICA vs. blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 2.4 samples need to be analysed per day.

Threshold analyses for IRIDICA versus matrix-absorbed laser desorption/ionisation time-of-flight mass spectrometry
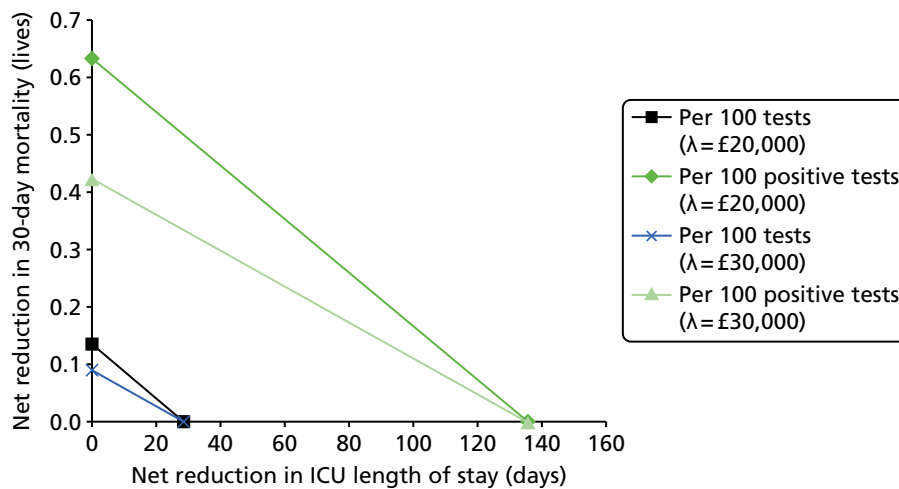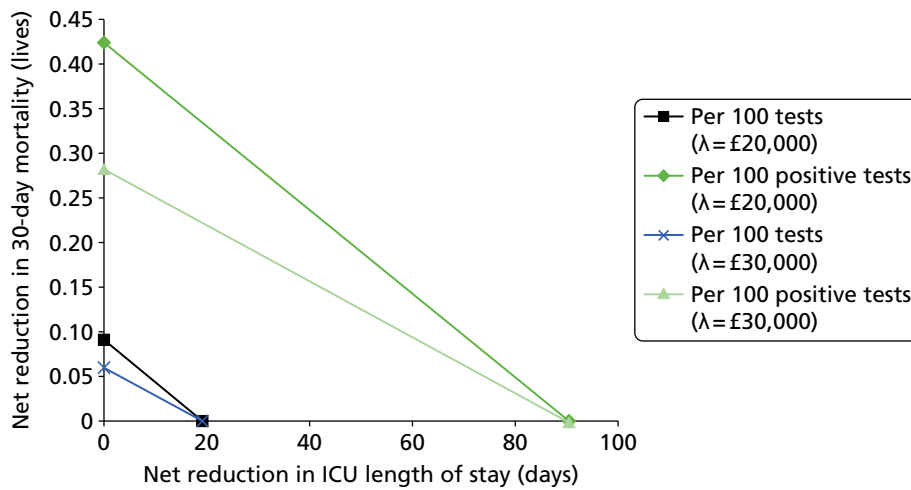

FIGURE 34 Threshold analyses for IRIDICA vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 2.4 samples need to be analysed per day.
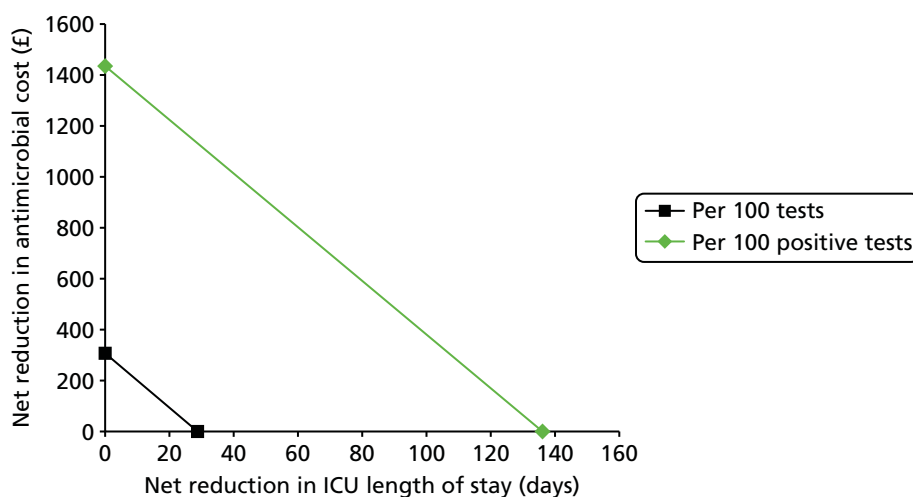
FIGURE 35 Threshold analyses for IRIDICA vs. MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 2.4 samples need to be analysed per day.

Assuming 17 samples a day require analysing

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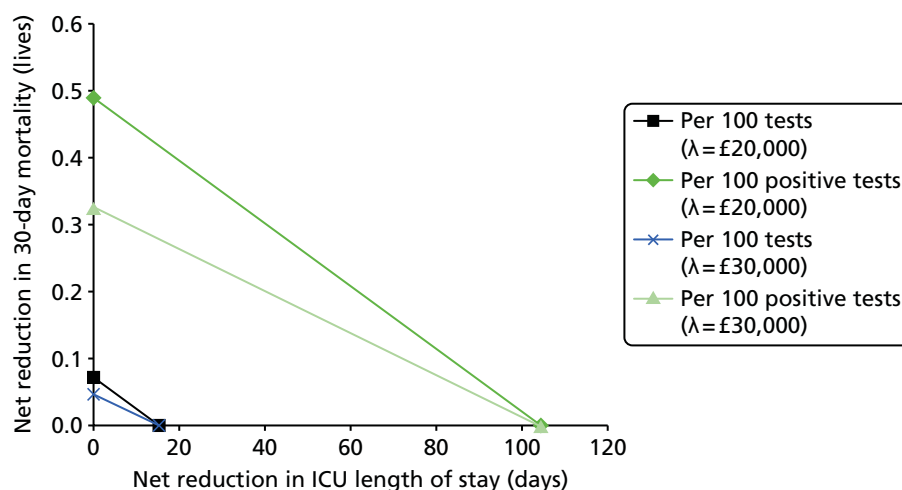
FIGURE 36 Threshold analyses for SeptiFast vs. blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 17 samples need to be analysed per day.

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

Threshold analyses for SeptiFast versus matrix-absorbed laser desorption/ ionisation time-of-flight mass spectrometry
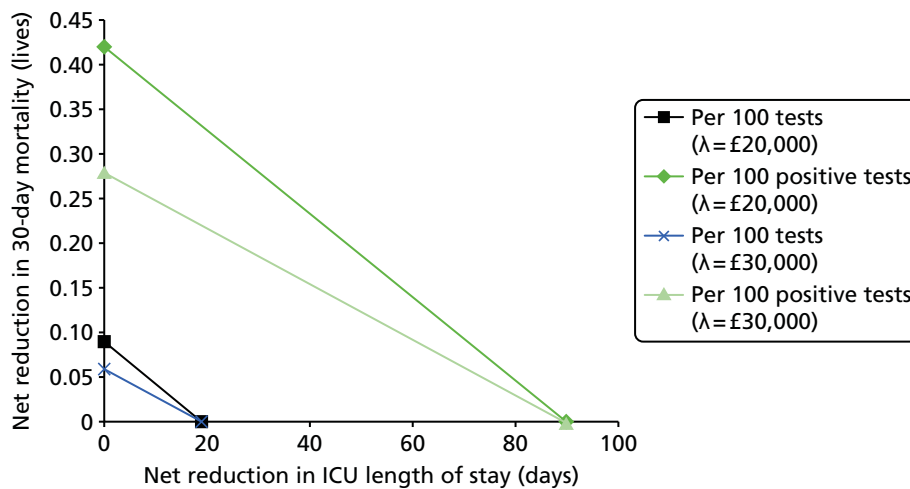

FIGURE 38 Threshold analyses for SeptiFast vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 17 samples need to be analysed per day.
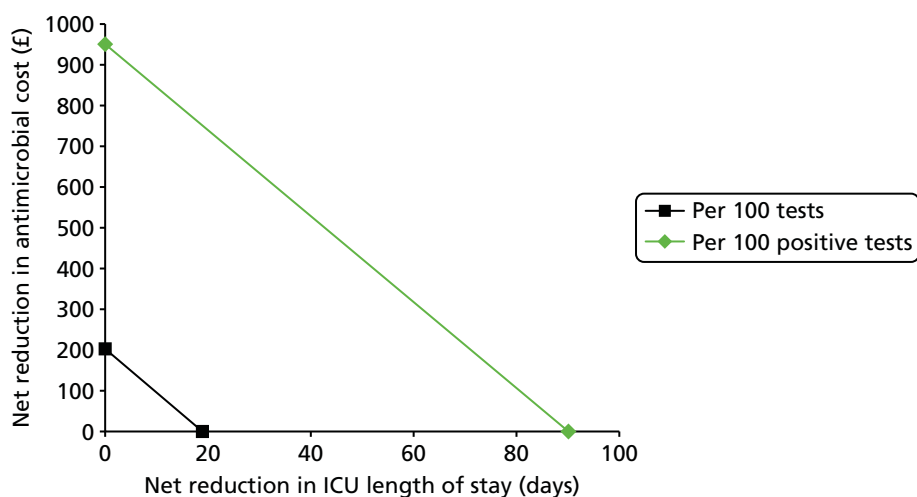

FIGURE 39 Threshold analyses for SeptiFast vs. MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 17 samples need to be analysed per day.

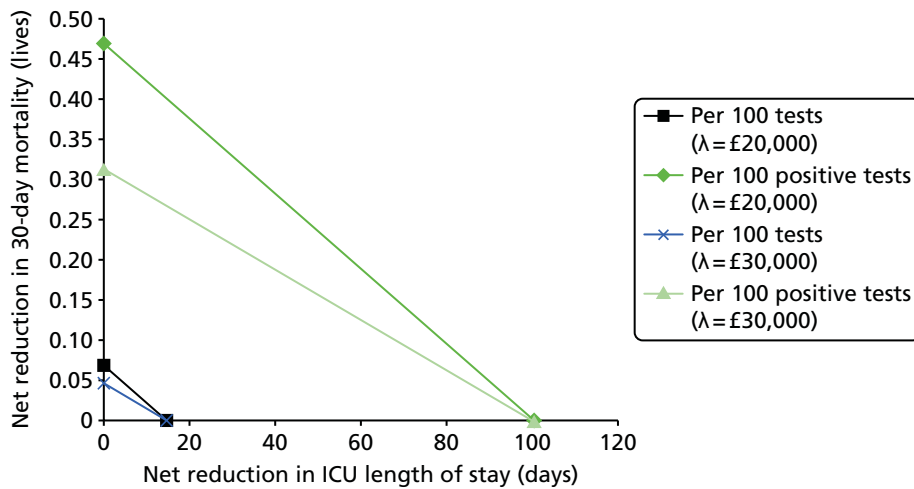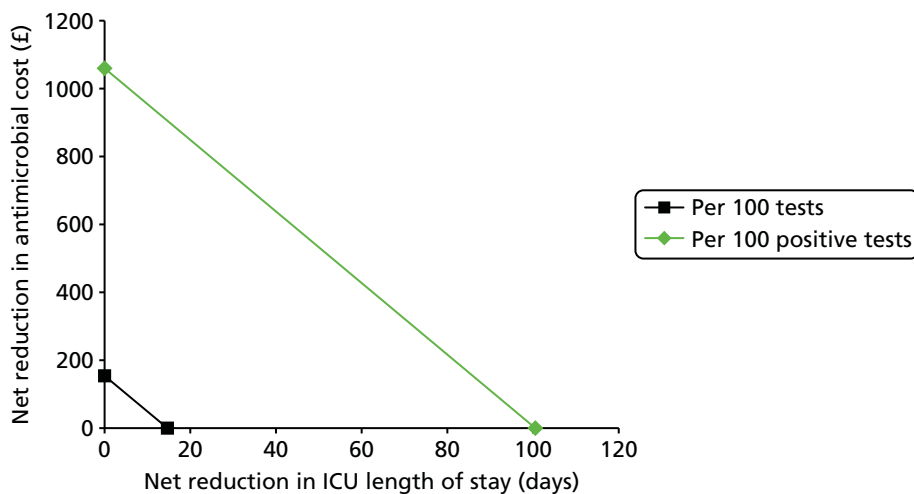### Threshold analyses for SepsiTest versus blood culture

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

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

Threshold analyses for SepsiTest versus matrix-absorbed laser desorption/ionisation time-of-flight mass spectrometry
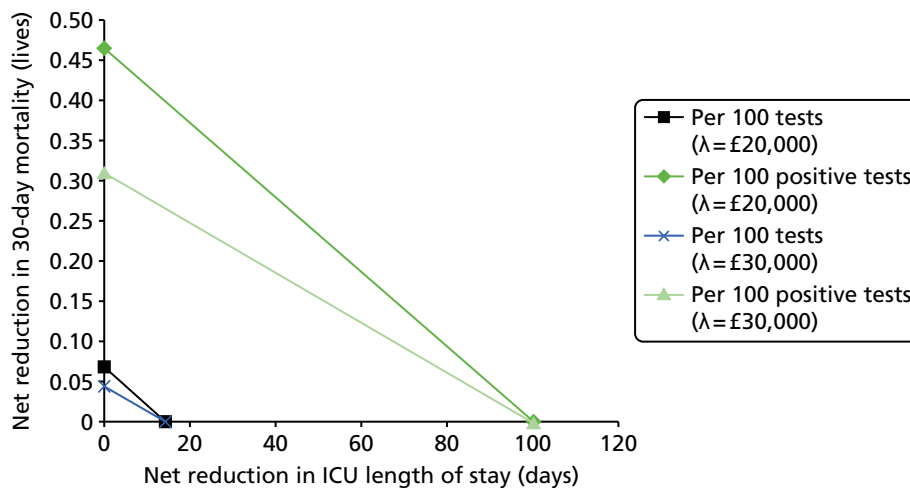

FIGURE 42 Threshold analyses for SepsiTest vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that machinery related to SepsiTest needs to be purchased and 17 samples need to be analysed per day.
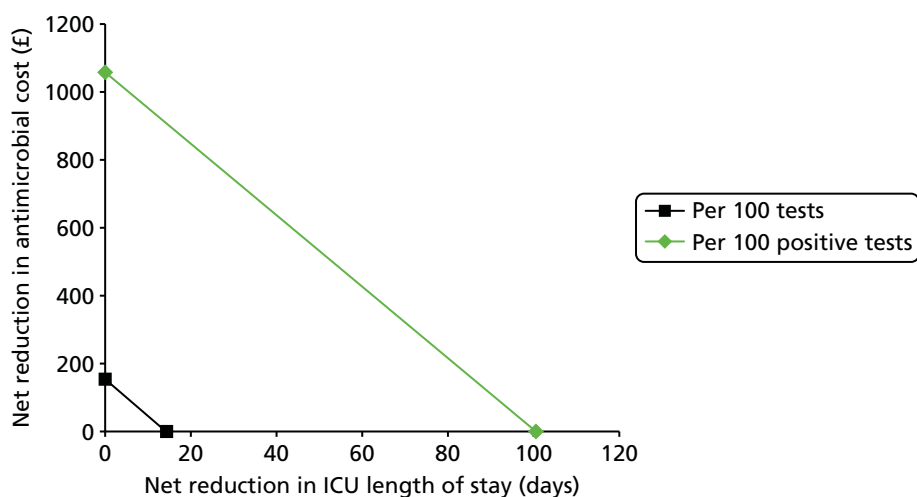

FIGURE 43 Threshold analyses for SepsiTest vs. MALDI-TOF MS using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that machinery related to SepsiTest needs to be purchased and 17 samples need to be analysed per day.

Threshold analyses for IRIDICA versus blood culture

FIGURE 44 Threshold analyses for IRIDICA vs. blood culture using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 17 samples need to be analysed per day.
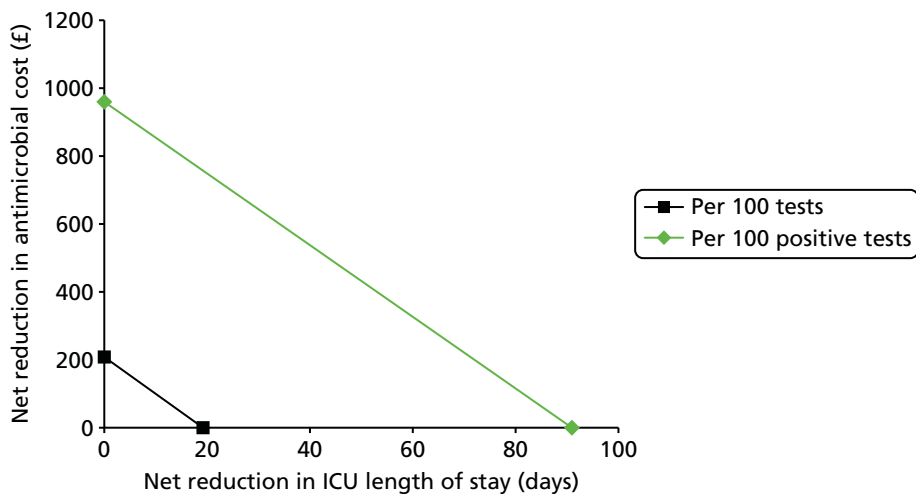
FIGURE 45 Threshold analyses for IRIDICA vs. blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 17 samples need to be analysed per day.

Threshold analyses for IRIDICA versus matrix-absorbed laser desorption/ ionisation time-of-flight mass spectrometry

FIGURE 46 Threshold analyses for IRIDICA vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 17 samples need to be analysed per day.

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

Assuming 68 samples a day require analysing

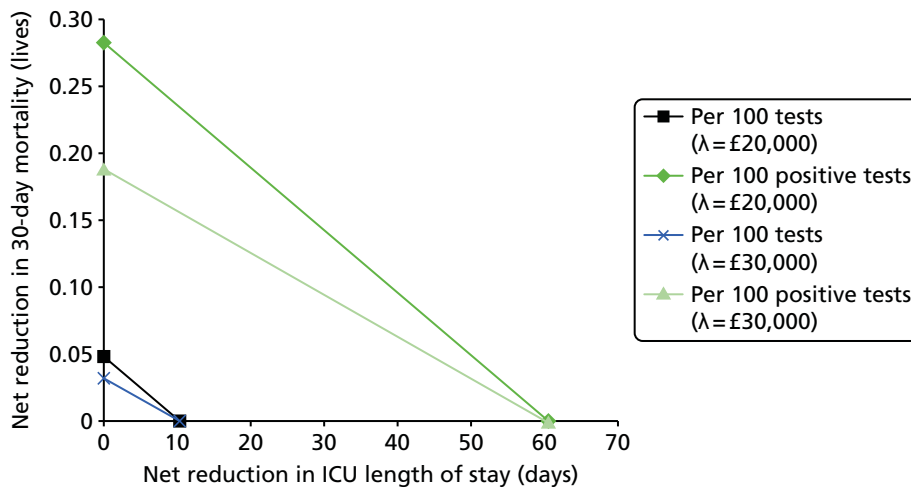*Threshold analyses for SeptiFast versus blood culture*

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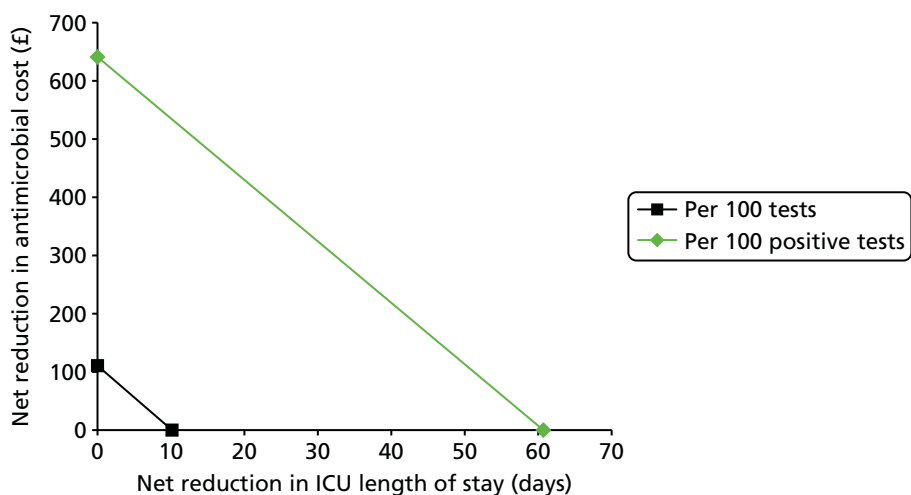

FIGURE 49 Threshold analyses for SeptiFast vs. blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 68 samples need to be analysed per day.

Threshold analyses for SeptiFast versus matrix-absorbed laser desorption/ionisation time-of-flight mass spectrometry
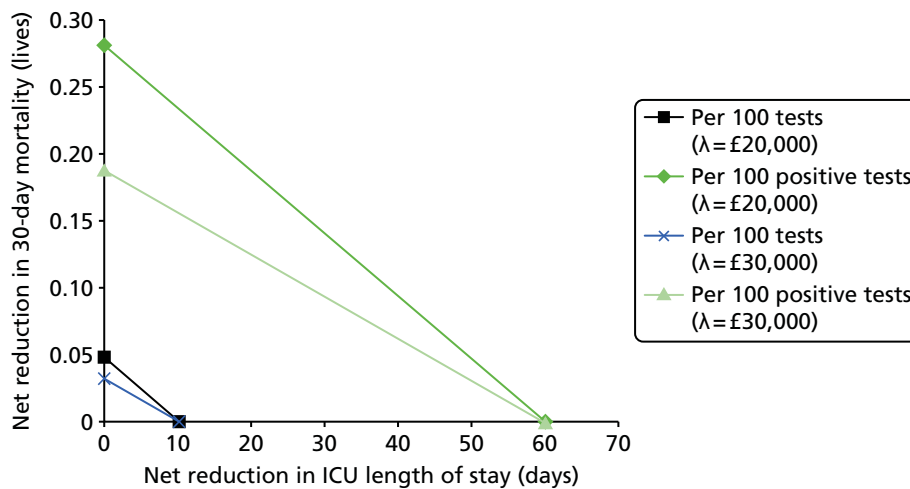

FIGURE 50 Threshold analyses for SeptiFast vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the SeptiFast machine needs to be purchased and 68 samples need to be analysed per day.

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

Threshold analyses for SepsiTest versus blood culture

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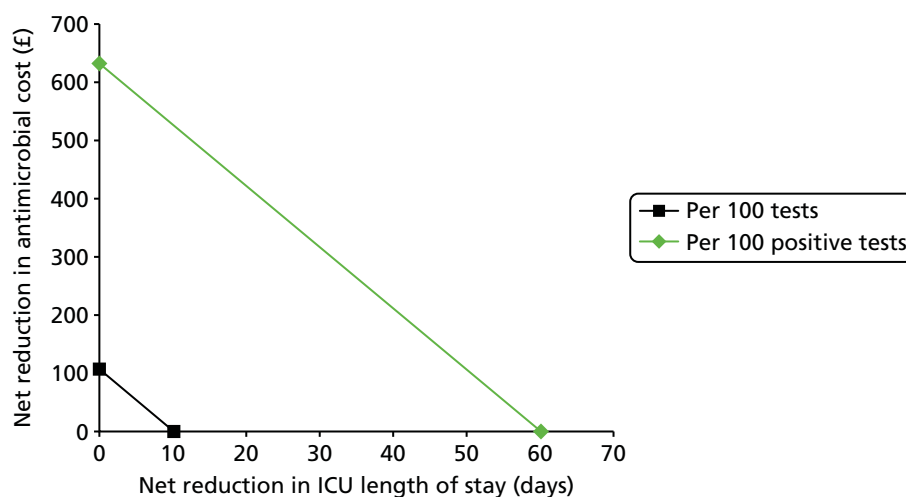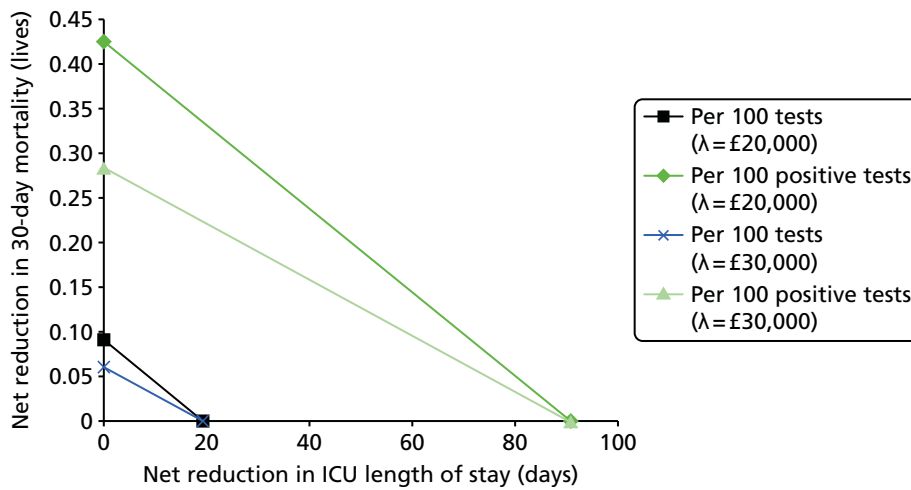

FIGURE 53 Threshold analyses for SepsiTest vs. blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that machinery related to SepsiTest needs to be purchased and 68 samples need to be analysed per day.

Threshold analyses for SepsiTest versus matrix-absorbed laser desorption/ ionisation time-of-flight mass spectrometry

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

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

Threshold analyses for IRIDICA versus blood culture

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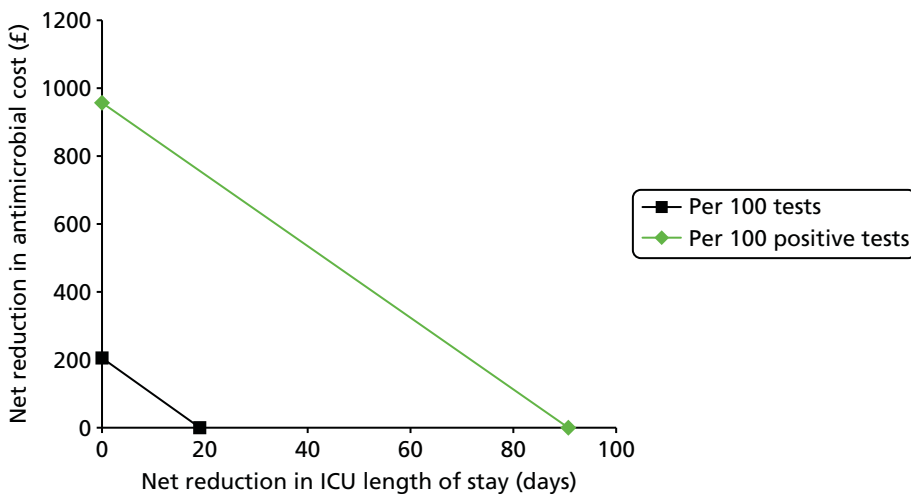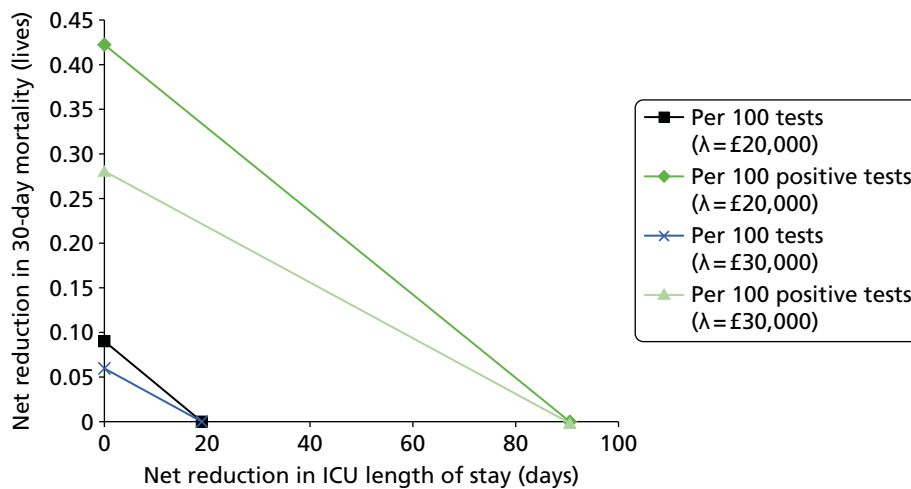

FIGURE 57 Threshold analyses for IRIDICA vs. blood culture using net reduction in antimicrobial treatment costs and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 68 samples need to be analysed per day.
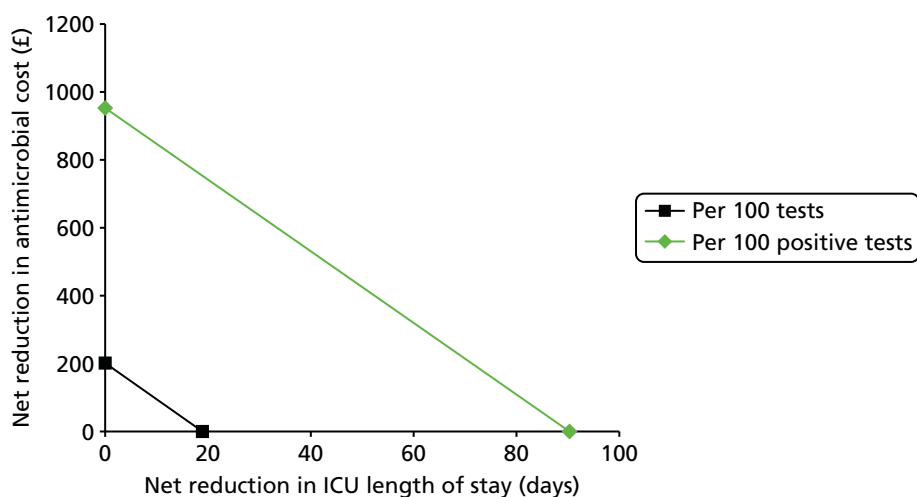
Threshold analyses for IRIDICA versus matrix-absorbed laser desorption/ ionisation time-of-flight mass spectrometry

FIGURE 58 Threshold analyses for IRIDICA vs. MALDI-TOF MS using net reduction in mortality and net reduction in ICU length of stay combined. Assuming that the IRIDICA machine needs to be purchased and 68 samples need to be analysed per day.

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

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