

Evidence Assessment and Analysis Report commissioned by the NIHR HTA Programme on behalf of the National Institute for Health and Care Excellence – Protocol

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1. Title of the project

The clinical and cost-effectiveness of 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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3. Plain English Summary

Sepsis is a common, potentially life threatening condition which is caused by an extreme reaction of the body's immune system to infection. Approximately 100,000 people are admitted to hospital due to sepsis each year and around a third of these patients die because of the sepsis episode.

Recognition of sepsis and rapid treatment with appropriate antimicrobial drugs is important to increase the chances of survival. If sepsis is considered possible, treatment, often including potent antibiotics usually reserved for more difficult to treat infections, is recommended to be started before the diagnosis has been confirmed to avoid potentially harmful delay. However, it is also important to distinguish between sepsis and whole body immune responses which are not caused by infection, so that the most appropriate treatment can be identified and antimicrobial treatment can be reduced. The reduction in the use of antimicrobial medications is needed to prevent these drugs being less effective and has been highlighted in Department of Health reports.

This project aims to evaluate three tests which potentially allow the rapid detection and identification of bacterial and fungal DNA present in the blood stream of people who are suspected of having sepsis. These tests are: the LightCycler SeptiFast Test MGRADE; SepsiTtest; and IRIDICA BAC BSI. Each test is intended to be run directly on whole blood samples without require prior incubation or pre-culture steps, allowing an earlier initial assessment of the patient. It is anticipated that blood cultures would be required in addition to each test to provide additional data on the most effective drug to use.

4. Decision problem

4.1 Purpose of the decision to be made

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 infection. Systemic illness often occurs when bacteria invade normally sterile parts of the body. One example of this is the invasion of bacteria or fungi into the blood stream, a process which often causes an inflammatory immune response.

If sepsis is not treated 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. 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
- Abnormal blood clotting

In the UK sepsis is estimated to be responsible for 100,000 hospital admissions and 37,000 deaths per year (Daniels 2011). Severe sepsis is one of the most common reasons for admission to a critical care unit, accounting for almost one third of all admissions. Severe sepsis is a time-critical condition where delays in recognition and the subsequent administration of appropriate treatment can adversely impact on outcomes.

Antimicrobial resistance describes the development of resistance to existing antimicrobial medications (including antibiotics, anti-fungals and anti-virals) amongst bacteria, viruses and fungi. As existing antimicrobial medications are becoming less effective, strategies such as the [UK five year antimicrobial resistance strategy](#) (Department of Health 2013) have been introduced to help conserve the effectiveness of existing treatments. One of the key priorities outlined in the UK five-year antimicrobial resistance strategy is the introduction of antimicrobial stewardship programmes which aim to promote the rational prescribing of antimicrobial medications and the use of existing and new rapid diagnostic tests.

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

4.2 Clear definition of the interventions

All interventions are used in conjunction with clinical assessment which would include blood culture, with or without matrix-absorbed laser desorption/ionization- time of flight (MALDI-TOF) mass spectrometry. It is anticipated that blood cultures would be required in addition to the rapid molecular tests to provide definitive antimicrobial susceptibility data where possible.

i. LightCycler SeptiFast Test MGRADE

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

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

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

The bacteria and fungi which can be detected by the LightCycler SeptiFast Test MGRADE are shown in Table 1.

Table 1: Bacteria and fungi detected by the LightCycler SeptiFast Test MGRADE

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

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

ii. SepsiTest

SepsiTest (Molzym Molecular Diagnostics) is a CE-marked PCR test for detecting bacterial and fungal DNA in 1ml K-EDTA-or citrate-treated whole blood. The test is able to identify species from more than 200 genera of bacteria and 65 genera of fungi, with the exception of *Candida krusei*.

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

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

iii. IRIDICA BAC BSI

The IRIDICA BAC BSI assay (Abbott Diagnostics) is a CE-marked in-vitro diagnostic test for detecting and identifying bacteria and candida DNA in 5ml EDTA-treated whole blood. The test can also detect the *mecA* (Staphylococcus specific methicillin resistance), *vanA* and *vanB* (Enterococcus specific vancomycin resistance) and *KPC* (gram-negative associated carbapenem resistance) genes which are associated with antibiotic resistance. The test is designed for use with the IRIDICA system which combines broad range PCR with electrospray ionisation time of flight mass spectrometry to amplify and detect pathogens. The IRIDICA analysis computer consists of a proprietary database and software which identifies the organism present in the sample by comparing the sequence of the sample with a library of known sequences. The IRIDICA BAC BSI system was developed from a previous version of the assay called PLEX-ID (Abbott).

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

4.3 Populations and relevant subgroups

Bacterial infections are the most common cause of sepsis and blood stream infection; however they can also be caused by viral and fungal infections. The most common sites of infection leading to sepsis are the lungs, urinary tract, abdomen and pelvis. Other sources of infection leading to sepsis include skin infections (such as cellulitis), post-surgical infections and infections of the nervous system (such as meningitis or encephalitis).

Patients who are currently or have recently been hospitalised, are at risk of acquiring a healthcare associated infection and are therefore at increased risk of sepsis and bloodstream infection. It is thought that the increasing number of invasive procedures (such as catheterisation), immunosuppressive therapy, antibiotic therapy and life support measures has resulted in an increase in healthcare associated blood stream infections (Public Health England 2014a). In 2011, a

total of 3,360 people in England were diagnosed with a healthcare associated infection, 255 (7.6%) of whom had a blood stream infection (Health Protection Agency 2012). Septic shock is most commonly associated with gram-negative bacterial blood stream infections, but shock can also be associated with blood stream infections caused by gram-positive bacteria, particularly with fulminant pneumococcal, Lancefield Group A streptococcal and staphylococcal infections (Public Health England 2014b). Community acquired blood stream infections may also occur in people who have not had recent contact with healthcare services. The pathogens isolated from these people may differ from those associated with hospital acquired blood stream infection (Public Health England 2014a).

Blood stream infection is also a risk for people who are immunocompromised, particularly amongst people with neutropenia, who are at risk of developing neutropenic sepsis. People who are immunocompromised often have a high incidence of infections caused by pathogens such as non-fermentative Gram-negative rods, *Listeria monocytogenes*, *Corynebacterium* species, *Candida* species, coagulase negative *Staphylococci*, *Enterococci* and *Viridans streptococci*. Polymicrobial infections are also more common amongst people who are immunocompromised (Public Health England 2014a).

The bacteria most commonly associated with bloodstream infection in adults in England, Wales and Northern Ireland are outlined below in Table 2.

Table 2: Bacteria species isolated from adults with bloodstream infections (Davies 2013)

Gram-negative		Gram-positive	
<i>Escherichia coli</i>	36%	<i>Staphylococcus aureus</i> (MSSA)	9.7%
<i>Klebsiella</i> spp.	7.8%	<i>Non-pyogenic streptococci</i>	7.1%
Other gram-negative	6.4%	<i>Enterococcus</i> spp.	6.3%
<i>Pseudomonas</i> spp.	4.3%	<i>Streptococcus pneumoniae</i>	4.2%
<i>Proteus</i> spp.	3.1%	Other gram-positive	4.2%
<i>Enterobacter</i> spp.	2.2%	<i>Staphylococcus aureus</i> (MRSA)	1.6%
<i>Bacteroides</i> spp.	1.5%	<i>Group B Streptococci</i>	1.4%
<i>Serratia</i> spp.	1.0%	<i>Group A Streptococci</i>	1.4%
<i>Acinetobacter</i> spp.	0.7%	<i>Diphtheroids</i>	1.2%

MSSA: methicillin-sensitive *staphylococcus aureus*; MRSA: methicillin resistant *staphylococcus aureus*.

The types of pathogens causing bloodstream infection can also differ in children compared to those isolated from adults with bloodstream infection. Pathogens known to cause community acquired

blood stream infection in children include *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Staphylococcus 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 healthcare associated infections in adults; however polymicrobial infection and anaerobic bacteraemia are thought to occur less frequently amongst children (Public Health England 2014a).

4.4 Place of the intervention in the treatment pathway(s)

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

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

The Commissioning for Quality and Innovation payment framework (CQUIN) which is currently in development have announced new sepsis mandates to monitor adherence to the sepsis care pathway across the NHS. 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.

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

- General variables: temperature of greater than 38.3°C or less than 36°C; heart rate greater than 90 beats per minute; rapid breathing, altered mental status; significant oedema; high blood sugar in the absence of diabetes.

- Inflammatory variables: low or high white blood cell count or more than 10% immature forms; raised plasma CRP; raised plasma procalcitonin.
- Haemodynamic and tissue perfusion variables: low blood pressure; raised blood lactate (a concentration of ≥ 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; raised plasma bilirubin levels.

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

- At least 2 sets of blood cultures should be collected (aerobic and anaerobic) before antimicrobial therapy is initiated if such cultures do not cause significant delay (>45 minutes) in the start of antimicrobial administration. At least one should be drawn percutaneously and one drawn through each vascular access device, unless the device was recently (<48 hours) inserted. The blood cultures can be drawn at the same time if they are obtained from different sites. Cultures of other sites such as urine cerebrospinal fluid, wounds, respiratory secretions or other bodily fluids that may be the source of infection should be obtained before initiation of antimicrobial therapy, if doing so does not cause significant delay in the start of antimicrobial administration.
- Imaging studies such as CT or X-ray should be performed in order to confirm a potential source of infection.
- Assays to diagnose systemic fungal infection should be used if available and invasive candidiasis is suspected.

4.5 *Relevant comparators*

The relevant comparators are variants of current standard care which consists of clinical assessment in conjunction with blood culture or clinical assessment in conjunction with blood culture and MALDI-TOF mass spectrometry. Blood culture is required for the potential identification of bloodstream bacteria and fungi, and to provide potential definitive antimicrobial susceptibility data.

[Standards for the investigation of blood cultures](#) are available from Public Health England (2014a). A blood culture set for the diagnosis of blood stream infection is defined as one aerobic and one anaerobic bottle (Public Health England 2014a). For adult patients it is recommended that 20-30ml of blood be cultured per set, and that two consecutive blood culture sets from two separate venepuncture sites should be collected during any 24hr period for each septic episode. The first set

should be taken prior to the administration of antimicrobial treatment as the presence of antibiotics or antifungals may inhibit the growth of pathogens in the blood culture (Public Health England 2014a). 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 system, which alert laboratory staff once growth has been detected.

When a blood culture 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 mass spectrometry.
- Identification should be followed by sensitivity testing to determine to which antimicrobials the identified pathogen is susceptible. If direct or automated sensitivity testing (including MALDI-TOF mass spectrometry) is used a report should be made within 24 hours, extended to 48 hours if traditional techniques such as the disc diffusion method are used.
- A preliminary positive report is made within 2 hours of identification and sensitivity testing, and a final positive report should be made within 5 days of the sample arriving in the laboratory (Public Health England 2014a). Discussions at the scoping workshop indicated that in some hospitals this process was being completed within 3 days.

If a blood culture is negative, it is recommended that a preliminary negative report is provided within 48 hours of sample receipt in the laboratory and a final negative report should be issued within 5 days unless extended culture is being undertaken for example if fungi or unusual, fastidious or slow growing organisms are suspected (Public Health England 2014a).

False negative blood culture results may occur due to the transient nature of blood stream infections and a low number of organisms present in each blood sample; often less than 1×10^3 colony forming units per litre in adults with blood stream infection (Public Health England 2014a). The presence of antibiotic treatment prior to the blood being extracted can also result in false negative results. Conversely, false positive blood culture results may occur when pathogens transferred from the skin during the drawing of blood contaminate the culture. To reduce the incidence of false positive results current standards recommended that contamination rates are no higher than 3% (Public Health England 2014a). In addition, several criteria are used to differentiate between contamination

and true blood stream infection which include: the identity and clinical significance of the pathogen; the number of positive blood culture sets and positive culture bottles; and the quantity of growth detected.

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

Issues to be considered regarding blood culture as a comparator

Blood culture is regarded as the reference standard; however, at the NICE scoping meeting (09.01.15) a number of limitations regarding its use were identified, for example:

- Blood culture does not always pick up fungal pathogens
- Blood culture tends to be negative around 60% of the time in an emergency department setting and 90% of the time in a critical care unit setting, indicating poor sensitivity which may be attributed to commencement of antimicrobial therapy prior to sample collection, low pathogen levels in blood, and inadequate blood sampling.

At the scoping workshop it was commented that there are now more downstream options to blood culture such as gram stain techniques and some hospitals are using in-house tests which are not CE-marked. These will not be included as comparators as they are neither used widely nor consistently across the NHS.

Management/treatment

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

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

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

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

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

It is noteworthy that the laboratory microbiology result is only one factor feeding into a clinical judgement which incorporates many different factors depending on the individual patient such as the patient's symptoms, their history and the results of other tests. Where appropriate, papers detailing latent class analyses will be considered.

4.6 *Key factors to be addressed*

Antimicrobial therapy

It is recommended that intravenous empiric antimicrobials should be administered within the first hour of recognition of septic shock and severe sepsis. The initial antimicrobial therapy should include one or more drugs that have activity against all likely pathogens (bacterial and/or fungal or viral) and that penetrate in adequate concentrations into the tissues presumed to be the source of sepsis (Surviving Sepsis Campaign 2012). Frequently used broad spectrum antibiotics for more serious infections include cephalosporins and aminoglycosides. Carbapenems are often the last option in patients with hard to treat infections (Department of Health 2013).

The choice of empirical antimicrobial therapy is often based on:

- the patient's history including drug intolerances
- receipt of antibiotics (previous 3 months)
- underlying disease
- the clinical syndrome
- susceptibility patterns of pathogens in the community and hospital
- previous microbiology reports identifying pathogens which have previously colonised or infected the patient

Clinicians should also consider whether fungi is a likely causative pathogen when selecting initial therapy and administer empirical antifungal therapy where necessary.

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

loss, damage to the kidneys and an increased risk of developing superinfection with *Clostridium difficile*.

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

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

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

5. Report methods for assessing the outcomes arising from the use of the interventions

Objectives

- Systematically review evidence for the clinical-effectiveness of the LightCycler SeptiFast Test MGRADE, SepsiTtest and IRIDICA BAC BSI assay in conjunction with clinical assessment for rapidly identifying bloodstream bacteria and fungi.
- Systematically review existing economic evaluations for the LightCycler SeptiFast Test MGRADE, SepsiTtest and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi.
- Develop a *de novo* economic model to assess the cost-effectiveness of the LightCycler SeptiFast Test MGRADE, SepsiTtest and IRIDICA BAC BSI assay in conjunction with clinical assessment for rapidly identifying bloodstream bacteria and fungi compared with clinical assessment which would include blood culture, with or without MALDI-TOF mass spectrometry

5.1 Population

The population will comprise people with suspected blood stream infections in secondary care who required blood cultures.

Potential subgroups will include the following:

- People with a suspected health care associated infection
- People with a suspected community acquired infection
- Children and neonates
- People who are immunocompromised
- People exposed to antibiotics prior to blood sample collection

These groups are not mutually exclusive.

5.1.1 Target condition

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

5.2 Interventions (Index Test)

The following interventions, in conjunction with clinical assessment, will be included:

- LightCycler SeptiFast Test MGRADE (Roche Diagnostics)
- SepsiTtest (Molzym Diagnostics)
- IRIDICA BAC BSI assay (Abbott) (previously PLEX-ID (Abbott))

5.3 Comparators (reference standard)

The reference standard will include current standard care and will include the following:

- Clinical assessment in conjunction with blood culture for the identification of bloodstream bacterial and fungal pathogens with or without MALDI-TOF

Given the CQUIN currently in development and the raft of clinical guidelines released in 2012 it will be assumed that standard practice is compliant with national guidelines.

5.4 Outcomes

Intermediate measures for consideration where evidence is available:

- Diagnostic accuracy
- Discordant results with blood culture
- Time to result
- Time to treatment
- Test failure rates
- Duration of ICU and/or hospital stay
- Duration of broad and narrow spectrum antimicrobial therapy
- Re-admission rate
- Change in antimicrobial treatment plan

Clinical outcomes for consideration where evidence is available:

- Side-effects associated with broad spectrum antimicrobial use
- Morbidity and mortality
- Severity of disease (as measured by scoring systems such as SOFA, SAPS II and APACHEII)
- Rates of superinfection (including *C. difficile*)
- Rates of resistant infections
- Health related quality of life

5.4.1 *Setting*

- Departments and wards providing care for acutely unwell patients
- Critical care units

Whilst global evidence will be included, evidence which most closely resembles the NHS is the main setting of interest.

5.5 *Study design*

Any clinical diagnostic accuracy study that compares the index test with standard culture results, with or without MALDI-TOF mass spectrometry on patients' whole blood samples during the management of suspected sepsis, or with any other named intervention, will be included.

Systematic reviews will be retrieved in order to check their reference lists for potentially relevant studies.

5.6 *Search strategy*

The search strategy will comprise the following main elements:

- Searching of electronic databases
- Contact with experts in the field
- Scrutiny of bibliographies of all retrieved papers
- Scrutiny of any company submissions

The following electronic databases will be searched and the search strategy will be adapted across the databases:

- MEDLINE:Ovid
- MEDLINE In-Process Citations and Other Non-Indexed Citations :Ovid
- EMBASE:Ovid
- Cochrane Database of Systematic Reviews (CDR): Wiley Online
- Cochrane Central Register of Controlled Trials (CENTRAL): Wiley Online
- Health Technology Assessment Database (HTA): Wiley Online
- Database of Abstracts of Reviews of Effects (DARE)): Wiley Online. 1995-2014
- NHS Economic Evaluation Database (NHS EED): Wiley Online. 1995-2014
- Science Citation Index Expanded: Web of Science

- Science Citation Index and Conference Proceedings Index: Web of Science
- Manufacturer and User Facility Device Experience (MAUDE): Internet
- MEDION database: Internet

Comprehensive electronic searches will be conducted to identify reports of published and ongoing studies on the diagnostic accuracy and cost effectiveness of: LightCycler SeptiFast Test MGRADE (Roche Diagnostics); SepsiTTest (Molzym Diagnostics); and IRIDICA BAC BSI assay (Abbott). Although none of the searches will be restricted by language, all searches will be limited by date from January 2006 due to the reasons noted below.

To date, all included rapid molecular tests (LightCycler SeptiFast Test MGRADE, SepsiTTest and IRIDICA BAC BSI 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 in 2006 (Raglio *et al.*, 2006) with subsequent full-text peer-reviewed publications appearing in 2008 (Mancini *et al.*, 2008 and Louie *et al.*, 2008). The SeptiFast test gained its CE mark in 2006. For the SepsiTTest assay, studies evaluating the use of SepsiTTest on whole blood samples in the clinical setting first appeared in 2008 (Disqué *et al.*, 2008) and 2009. (Wellinghausen *et al.*, 2009) It received a CE mark in 2008. The IRIDICA platform received a CE mark in 2014 and has been available for purchase by the NHS since 16th November 2014. The previous version of the IRIDICA system was referred to as PLEX-ID (earlier versions were known as TIGER and Ibis T5000). However, the published evidence base for the PLEX-ID technology was originally based on positive blood cultures (Jordana-Lluch *et al.*, 2010; Kaleta *et al.*, 2011a; Jeng *et al.*, 2012; Kaleta *et al.*, 2011b). In 2013, the first clinical studies on whole blood samples using this technology for the diagnosis of bloodstream infections was published by Jordana-Lluch *et al.*, 2013 and Laffler *et al.*, 2013, although at the time of writing it was unclear if PLEX-ID had received a CE mark for such usage. PLEX-ID has now been superseded by the IRIDICA platform.

The search strategy of a recent and relevant systematic review on SeptiFast (Dark *et al.*, 2015) was initially used and amended to include the following (see Appendix 1):

- generic, trademark or other product names of all the relevant interventions
- bacterial or fungal genes concept to be combined with PCR and population terms
- a limit to exclude all only animal studies

Scoping searches with the amended search strategy have shown that the included and published studies reported in Dark *et al.*, (2015) and those mentioned above (for IRIDICA and SepsiT_{est}) were retrieved.

Additionally completed and ongoing trials will be identified by searches of the following registries:

- NIH ClinicalTrials.gov (<http://www.clinicaltrials.gov/>)
- Current Controlled Trials (<http://www.controlled-trials.com/>)
- WHO International Clinical Trials Registry Platform (ICTRP) (<http://www.who.int/ictrp/en/>)

Relevant websites of key professional organisations and of testing devices companies will be checked for additional information.

Additional searches on the outcomes to inform the decision analytical model will also be undertaken, where required in the course of the project.

All Identified references will be downloaded in a bibliographic database for further assessment and handling. References in retrieved articles will be checked for additional studies.

- Inclusion criteria

Included studies that meet the above criteria (i.e. population, target condition, index test, reference standard, setting and outcomes) will be restricted to those published in the English language. Both full text papers and conference abstracts published from 2006 onwards will be sought.

- Exclusion criteria

Reviews of primary studies will not be included in the analysis, but will be retained for discussion and identification of additional studies. Moreover, the following publication types will be excluded from the review

- Biological studies
- Narrative reviews, editorials and opinions
- Case reports
- Non-English language reports
- Reports published as meeting abstracts only, where insufficient methodological details are reported to allow critical appraisal of study quality

Details of all full text excluded papers (including non-English language citation) will also be provided in the review.

5.7 *Data extraction strategy*

The selection of potentially relevant articles will be undertaken using a two-step process. First all titles will be examined for inclusion by one reviewer (any citations that clearly do not meet the inclusion criteria i.e. non-human, unrelated to sepsis will be excluded). Second, all abstracts and full text articles will be examined independently by two reviewers. Any disagreements will be resolved by consensus or arbitration by a third party.

A data extraction form will be developed and piloted for the purpose of this assessment. One reviewer will extract information on study design, characteristics of participants, settings, characteristics of interventions, alternative interventions and comparators, and outcome measures as described above. A second reviewer will check the data extraction. Any disagreements will be resolved by consensus or arbitration by a third party.

Unpublished study data from the company that meet the inclusion criteria which are received during the review process, will be extracted and quality assessed in accordance with the procedures outlined in this protocol.

Where multiple publications of the same study are identified, data will be extracted and reported as a single study. Moreover, as this review of three rapid molecular tests incorporates an update of the most recent review of SeptiFast (Dark *et al.*, 2014), all relevant data will be extracted from the systematic review in the first instance, but will be cross checked for accuracy with the original papers. If necessary, additional data will be extracted from the original papers. For the review of Sepsitest and IRIDICA, all data will be extracted from the original papers.

5.8 *Quality assessment strategy*

The methodological quality of each included study will be assessed according to (adapted) criteria based on those proposed by Whiting *et al.*, (2011).

A single reviewer will assess the methodological quality of the included studies and findings checked by a second reviewer. Any disagreements will be resolved by consensus or arbitration by a third party.

5.9 Methods of analysis/synthesis

The External Assessment Group (EAG) have experience of multiple statistical techniques: the most appropriate will be chosen to address the data within the decision problem.

Where evidence is available, sensitivity analyses will be performed including only:

- Those studies at a low risk of bias
- People with a suspected health care associated infection.
- People with a suspected community acquired infection
- Children and neonates
- People who are immunocompromised

6. Report methods for synthesising evidence of cost effectiveness

6.1 Identifying and systematically reviewing published cost-effectiveness studies

A systematic review will be undertaken to identify any published evaluations of the cost-effectiveness of tests for diagnosis of sepsis. In order to constrain the number of papers identified whilst keeping those deemed relevant the literature review will identify only papers published since 2006. Any full papers identified will be appraised.

6.2 Development of a health economic model

It is likely that there will be no published models that can be used to address the decision problem; therefore a *de novo* mathematical model will be developed. The final metric of cost-effectiveness will be cost per QALY gained if that is deemed plausibly robust. The time horizon of the model will be patient lifetime in order that any differential mortality or morbidity associated with interventions and comparators are fully considered. The model will be in line with recommendations made within the NICE reference case (NICE 2011) where possible,

The EAG is familiar with multiple modelling techniques and will not finalise the method until the data have been reviewed although it is likely that major simplifications would be needed, or threshold levels cited, to incorporate the complexity of issues within the decision problem. An example of excessive complexity would be in attempting to robustly establish the benefits of antibiotic stewardship: a model seeking to evaluate the impacts throughout the country of an individual receiving antibiotics for a fewer number of days would be time consuming to construct and would also produce highly uncertain results. The following, non-exhaustive, lists of features will be acknowledged within the modelling.

The potential benefits of the interventions in:

- Improvement of patient outcomes and the cost implications (if data are available to suggest that the effectiveness of a focussed antibiotic is greater than the antibiotic within the broad spectrum package)
- Improvement in the adverse event profile from prescribing fewer antibiotics
- Cost-savings from prescribing fewer antibiotics
- Indirect health gains through better antibiotic stewardship
- Direct health gains through reduced risk of superinfection or complications associated with a secondary incident post-sepsis

- Indirect health gain through a definitive diagnosis of alternative disease where sepsis is not present

The potential disadvantages of the interventions in:

- Increasing the costs associated with treating sepsis
- Inappropriate de-escalation of antibiotics where more than one infection is present yet the intervention identifies only one strain

Reviews of published literature or elicitation of the opinions of clinical experts will be considered to address the listed potential advantages and disadvantages.

Utility values for conditions included in the final model will be taken from published literature where available. Costs will be considered from an NHS and Personal Social Services perspective. Data for the cost analyses will be drawn from routine NHS sources (e.g. NHS reference costs, Personal Social Services Research Unit (PSSRU) and the British National Formulary (BNF)).

Where appropriate, discussions with individual hospitals and with the companies producing the interventions will be undertaken to produce more precise estimates of both acquisition, maintenance and running costs of interventions and comparators in addition to the potential costs associated with staff training. Furthermore, costs associated with treatment and from inappropriate treatment due to false test results will be considered. If necessary, costs of service reconfiguration will be estimated.

Where appropriate, multiple models will be developed in order to estimate the cost-effectiveness of the interventions in subgroups of the population. This will be undertaken where there is a facet of the population or treatment pathway that differs from the remaining population and that is likely to impact on the estimated cost per QALY gained or the threshold level of QALYs required for an intervention to achieve a stated cost per QALY value.

Explicit consideration will be given to the fact that blood culture may not be a perfect gold standard.

7. Handling of information from the companies

Data submitted by the companies will be considered if received by the EAG no later than 01/03/2015: If the data meet the inclusion criteria for the review they will be extracted and quality assessed in accordance with the procedures outlined in this protocol.

Any 'commercial in confidence' data provided by the companies, and specified as such, will be highlighted in blue and underlined in the assessment report (followed by company name in parentheses). Any 'academic in confidence' data provided by the companies, and specified as such, will be highlighted in yellow and underlined in the assessment report. Any confidential data used in the cost-effectiveness model will also be highlighted.

8. Competing interests of authors

The authors have no conflicts of interest to declare.

9. Timetable/milestones

Milestones	Completion date
Draft protocol	13 th January 2015
Final protocol	4 th February 2015
Progress report	6 th May 2015
Draft assessment report	2 nd July 2015
Final assessment report	30 th July 2015
Final executable economic model	3 rd August 2015

10. References

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11. Appendices

Appendix 1 Draft 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"