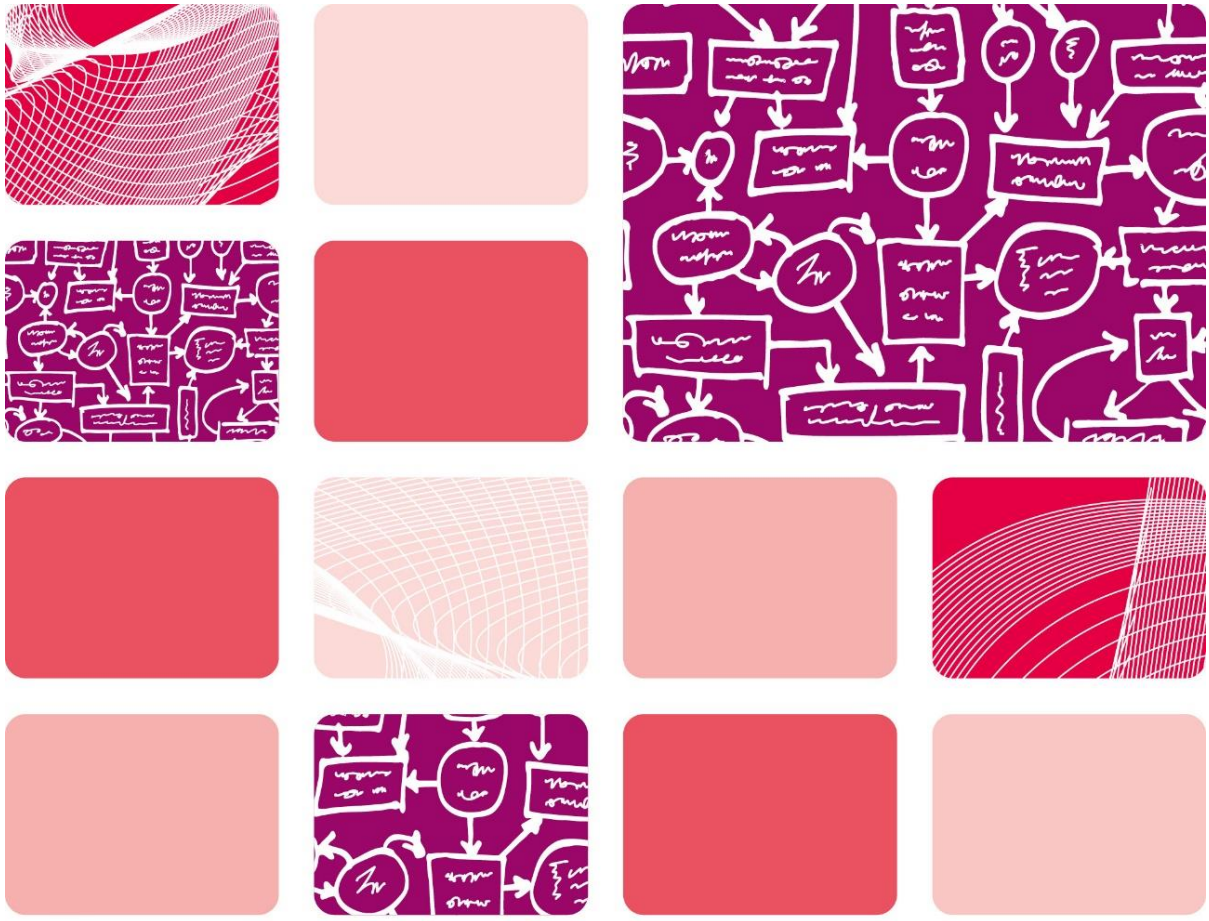
UK Health Security Agency

UK Standards for Microbiology Investigations

Sepsis and systemic or disseminated infections



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	1/24.04.25
Issue number discarded	1
Insert issue number	1.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 31.01.2023.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.
Section 10: Public health responsibilities of diagnostic laboratories	This section has been added to UK SMI templates to highlight the public health responsibilities that diagnostic laboratories have as part of their duties.

Amendment number/date	-/31.01.2023
Issue number discarded	-
Insert issue number	1
Anticipated next review date*	31.01.2026
Section(s) involved	**Amendment**
Whole document	This new syndromic document is based on *UK SMI B 37: Investigation of blood cultures (for organisms other than Mycobacterium species)*. The content and scope have expanded, and the document is presented in a new template with the relevant titles and headings.

*Reviews can be extended up to 5 years where appropriate.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This document describes the initial investigations that should be considered with different presentations of sepsis and systemic or disseminated infections caused by bacteria, viruses or fungi.

The UK SMI does not address the detection of parasites, *Mycobacterium* species or viral load testing and it does not list specific details of kits or platforms.

This UK SMI should be used in conjunction with other UK SMIs. Investigations to identify the source and cause of these infections and their dissemination are covered in the following documents:

UK SMI B 17 – Tissues and biopsies from deep seated sites and organs

UK SMI B 20 – Investigation of intravascular cannulae and associated specimens

UK SMI B 22 – Investigation of cerebrospinal fluid shunts

UK SMI B 27 – Investigation of cerebrospinal fluid

UK SMI B 31 – Investigation of specimens other than blood for parasites

UK SMI B 38 – Investigation of bone marrow

UK SMI B 39 – Investigation of dermatological specimens for superficial mycoses

UK SMI B 40 – Investigation of specimens for Mycobacterium species

UK SMI B 41 – Investigation of urine

UK SMI B 42 – Investigation of bone and soft tissue associated with osteomyelitis

UK SMI B 44 – Investigation of prosthetic joint infection samples

UK SMI B 57 – Investigation of bronchoalveolar lavage, sputum and associated specimens

UK SMI V 11 – HIV screening and confirmation

UK SMI V 28 – Investigation of cytomegalovirus infection.

UK SMI V 43 – Investigation of viral encephalitis

UK SMI V 44 – Investigation of orthopaedic implant associated infections

The UK SMIs focuses on established, validated and accredited methods available to laboratories. Novel technologies are also reviewed and included when sufficient evidence for their performance is available. Specific products are not reviewed.

Please note, following the recent update of fungal taxonomy, many species formerly part of the genus Candida now belong to a number of other genera. For the purposes of this document, both old and new names are mentioned as required and they are collectively referred to as '*Candida* and associated ascomycetous yeasts' (1).

4 Background

Sepsis and systemic or disseminated infections represent a spectrum of clinically highly significant disease, requiring a wide diagnostic lens. The detection and identification of micro-organisms from blood is essential for microbiological diagnosis of bacteraemia, fungaemia (particularly candidaemia), infective endocarditis and conditions associated with a clinical presentation of pyrexia of unknown origin. However, the investigation of samples from other sterile sites (cerebrospinal fluid, bone, other tissues) or deep sites (bronchoalveolar lavage, suprapubic aspirate urine) relevant to the presentation is critically important for identifying the source of the infection as well as the extent of its spread. Blood culture is also important for the diagnosis of prosthetic device infections such as vascular grafts and vascular access device associated sepsis. Blood cultures may also detect infections associated with other syndromes such as pneumonia, urinary tract and intra-abdominal infections. In some clinical settings, namely critically ill and significantly immunocompromised patients, targeted investigation for certain viral and fungal causes is indicated.

Antimicrobial resistance amongst pathogens (particularly Gram negative bacteria) is an important cause of ineffective empirical treatment of bloodstream infections (2,3). Early identification and antibiotic susceptibility results for blood culture isolates provide valuable diagnostic information. Appropriate antimicrobial therapy can be administered, which will reduce morbidity and mortality, improve patient care, support antimicrobial stewardship and reduce healthcare costs (4-6). Decreasing turnaround times (TAT) at each stage of the process from transportation of samples to reporting of results is therefore recommended (7).

Increasing numbers of kits and platforms are available that can detect specific bacterial, viral and fungal pathogens and their important resistance mechanisms more rapidly than traditional technologies.

4.1 Bloodstream infection

Bloodstream infection is caused by bacteria (bacteraemia), fungi (fungaemia) or viruses (viraemia) in the blood and may be transient, intermittent or continuous (8,9).

Microorganisms may enter the bloodstream from a focus of infection within the body, a colonised surface site through broken skin or mucous membrane, the gastrointestinal tract, by the direct introduction of contaminated material to the vascular system or by viral reactivation (10). These microorganisms are normally removed from the bloodstream within

a few minutes, however; systemic infection can result if the host defences are overwhelmed or evaded. Mortality depends on the type of infecting organism and the nature of any underlying disease (11,12).

4.1.1 Transient

The transient presence of bacteria, fungi or virus in the bloodstream for periods of several minutes may follow manipulation of, or surgical procedures involving infected tissue or the instrumentation of colonised mucosal surfaces. Common examples include dental extraction and urinary catheterisation.

4.1.2 Intermittent

Intermittent infection is characterised by the transient presence of bacteria, fungi or virus associated with undrained abscesses or biofilms and occurs in a variety of systemic and localised infections. Cultures taken during fevers and after the onset of rigors may miss intermittent bacteraemia or fungaemia as these tend to be cleared by the host defence mechanisms prior to sampling. Increasing the volume of blood will increase the chances of detecting intermittent and low-grade bacteraemia (13).

4.1.3 Continuous

Continuous bacteraemia, fungaemia or viraemia (dependent on the viral load) suggests a severe infection that has overwhelmed the host defence. It is also characteristic of intravascular infection such as infective endocarditis or suppurative thrombophlebitis. Continuous bacteraemia may also be a result of line related infection or contamination.

4.1.4 Pseudobacteraemia and pseudofungaemia

Blood culture contamination may occur at any stage between taking a blood sample and processing in the laboratory; when blood culture isolates originate from outside the patient's bloodstream this is termed pseudobacteraemia. Outbreaks of pseudobacteraemia (14,15) and pseudofungaemia (16) with environmental organisms have been described involving contaminated equipment on wards and laboratories.

4.2 Deep seated infection

A deep seated infection can be the focus of a bloodstream infection or a consequence of it. In either case, these involve sites, organs or tissue that cannot be reached without an invasive procedure to obtain samples. The main sample types for investigating these infections are surgical samples, tissue and needle biopsies and tissue fluid samples (CSF, synovial fluid, vitreous fluid). With the increasing sophistication of clinical imaging and sampling devices there are few organs in the human body that cannot be biopsied.

All these specimens are precious and in addition to bacterial infection, should be investigated for fungi, *Mycobacterium* species and parasites. Tissue obtained at operation is particularly precious as the sampling procedure may not be repeatable. Ideally these specimens should be discussed with the laboratory prior to sampling to ensure that transport and processing are timely and appropriate tests are performed. Prolonged storage (1 month) of residual specimens may be critical in enabling the arrangement of any further appropriate investigations such as mycobacterial cultures or referral for 16S or 18S rDNA PCR.

Histological investigation will often inform the decision to investigate for particular classes of infection. For instance, the presence of caseating granulomata should raise the suspicion of tuberculous infection; similar appearances may be caused by deep fungal infection on occasion. Good communication between the histopathology and the microbiology laboratories is important in this.

For more detail about the investigation of specific tissue samples please see UK SMI B 17 – Tissues and biopsies from deep seated sites and organs.

4.3 Disseminated infection

Disseminated infection occurs when a microorganism causes widespread infection in the body, affecting multiple organs, tissues and sites. Bloodstream infection may be transient or not detectable in disseminated infection. Therefore, in the presence of disseminated infection it is important to investigate as many sites, organs and tissues as possible, including the blood.

4.4 Sepsis

4.4.1 Sepsis and septic shock

Sepsis is defined as life threatening organ dysfunction caused by a dysregulated host response to infection (17,18). Septic shock is a subset of sepsis in which particularly profound circulatory, cellular and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone (19).

4.4.2 Neonatal sepsis

Neonatal sepsis may occur within the first 28 days of life (20). The incidence of neonatal sepsis increases with low birth weight or prematurity and can be divided into early onset (within 72 hours of birth) and late onset (after 72 hours) (21). Early onset sepsis (EOS) is the result of vertical bacterial transmission from the mother during the perinatal period occurring less than 72 hours after birth. Late onset sepsis (LOS), in contrast, results from postnatal

environmental exposure to pathogenic bacteria occurring 72 hours or more after birth (22,23).

Organisms isolated from all specimens taken for screening neonates including gastric aspirate and amniotic fluid may indicate colonisation and may include pathogens responsible for neonatal sepsis. However, they do not establish the presence of active systemic infection.

4.5 Organisms implicated

4.5.1 Community acquired infection

Community acquired bacteraemia and occasionally, fungaemia may arise in previously healthy individuals, usually in association with demonstrable focal infection such as pneumococcal pneumonia or significant trauma. Bacteria may also enter the blood from the patient's own commensal flora or from an undetected infected site and cause metastatic infection (for example, as may occur following *Staphylococcus aureus* bacteraemia). Other generalised bacteraemia illnesses include enteric fever (for example typhoid) and brucellosis.

Commonly isolated organisms include:

- *Escherichia coli* and other Enterobacterales
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- β-haemolytic streptococci, particularly *Streptococcus pyogenes*

Zoonoses are infections that can pass between animals and humans. These are less common but yet significant causes of community acquired infection. Organisms associated with zoonoses include *Pasteurella* species, *Capnocytophaga* species and *Brucella* species.

4.5.2 Healthcare associated infection (HCAI)

HCAI is infection that results from healthcare or treatment provided in any setting (24).

Invasive procedures such as catheterisation may introduce organisms to the bloodstream, while interventions such as immunosuppressive therapy or antibiotic therapy may weaken host defences. Organisms most frequently isolated depend on the care setting and patient group and may change with the duration of stay in hospital.

Organisms include (25,26):

- *E. coli* and other Enterobacterales
- *S. aureus*
- Coagulase negative staphylococci
- *Pseudomonas aeruginosa*
- Enterococci
- Anaerobes

- *Candida* and associated ascomycetous yeasts

Many other organisms have been implicated in both hospital and community acquired bacteraemia (27,28).

4.5.3 Pregnancy

In addition to other common causes of bacteraemia, *Listeria monocytogenes* may cause serious infection in pregnancy. Sepsis caused by *L. monocytogenes* presents as an acute febrile illness that may affect the foetus (29). This may lead to systemic infection (granulomatosis infantisepticum), stillbirth or neonatal meningitis. Products of conception, placenta and neonatal screening swabs should be examined for this organism. Routine culture of vaginal swabs for *L. monocytogenes* is not usually performed but may be useful in suspected cases (29).

Septic spontaneous abortion may result in serious maternal morbidity and may be fatal. Uterine perforation, presence of necrotic debris and retained placental products can all lead to infection; most infections are polymicrobial and involve anaerobes. Clostridial sepsis complicating abortion is potentially lethal. *Clostridium* species are part of the normal vaginal flora in some women.

4.5.4 Neonates

Organisms associated with neonatal sepsis include (20,21):

- β-haemolytic streptococci, in particular Lancefield group B streptococci
- *E. coli* and other Enterobacterales
- *S. aureus*
- Coagulase negative staphylococci
- *Listeria monocytogenes*
- *Enterococcus* species
- Pseudomonads
- *Candida* and associated ascomycetous yeasts
- Herpes simplex virus
- Enteroviruses

Group B streptococci and *E. coli* are major causes of early onset neonatal sepsis (30).

Neonatal sepsis caused by anaerobic bacteria have been reported; the majority of cases due to *Bacteroides* species, *Clostridium* species or *Peptostreptococcus* species (31).

4.5.5 Children

Organisms most commonly isolated from children with community acquired bacteraemia include:

- *S. pneumoniae*
- *N. meningitidis*
- *S. aureus*

- *E. coli*

Organisms implicated in nosocomial infections in children are similar to those seen in adults; polymicrobial and anaerobic bacteraemia however, occur less frequently (31).

Transient bacteraemia can occur in children with few or none of the symptoms normally associated with bloodstream infection. *S. pneumoniae* predominates, but occult infection with *H. influenzae*, *Salmonella* species and *N. meningitidis* has also been described (32). In patients who are immunocompromised, infections caused by less virulent organisms of the normal host and environmental flora such as fungi are common causes of blood stream infections.

4.5.6 Infective endocarditis (IE)

IE is defined as an infection of the heart valves or other areas of the endocardium. Infection can occur with both native valves and prosthetic valves (33). The Duke criteria is used for diagnosis (34). Historically, the disease was classified as either ‘acute’ or ‘subacute’; however, it is more usual to describe the disease in relation to the infecting organism or the underlying anatomy (35). Endocarditis caused by organisms that cannot be easily cultured, for example *C. burnetii*, can be detected in blood culture.

Native valve endocarditis

Chronic sources of bacteraemia in conjunction with common predisposing factors include congenital heart disease, mitral valve prolapse, and degenerative valvular disease in the elderly. Infective endocarditis can occur on anatomically and functionally normal valves as a result of certain bacteraemia.

Organisms most commonly isolated include (36):

- Oral streptococci
- Staphylococci (especially *S. aureus*)
- Enterococci
- *S. gallolyticus subsp. gallolyticus (*may also be referred to as *S. bovis* biotype 1)

Many other organisms have been described, including some that are fastidious, and that rarely cause human disease other than endocarditis (for example the HACEK group: *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium* species, *Eikenella corrodens* and *Kingella* species (see UK SMI ID 12 — Identification of Haemophilus species and the HACEK group of organisms). The utility of extended blood culture incubation for these organisms has been investigated; several studies have shown that extended incubation is unnecessary when using continuous monitoring blood culture analyser (37,38). *Bartonella* species have been implicated as causes of endocarditis with severity of infection correlating with immune status (39). Fungal endocarditis is rare, except in people who inject drugs, who have prolonged candidaemia and who have severe underlying illnesses and requires immediate treatment or surgery (40,41).

Prosthetic valve endocarditis (PVE)

The incidence of PVE in the first year after valve surgery is between 1 and 5%, decreasing to 1% after a year (36). IE involving the prosthetic aortic valve is more common than that involving prosthetic mitral, tricuspid or pulmonary valves (42).

'Early' PVE usually occurs within 60 days of implantation, but illness characteristic of early disease may not become apparent until 4 to 6 months after valve replacement. The causative organisms of early PVE are often more resistant to antibiotics than those for 'late' PVE which are more similar to those implicated in native valve endocarditis (35). Late PVE may occur several years after valve implantation and is associated with a lower mortality rate than early PVE.

The most commonly isolated organisms are (36):

- coagulase negative staphylococci
- *S. aureus*
- Gram negative bacilli
- *Candida* and associated ascomycetous yeasts
- Streptococci and enterococci
- *Corynebacterium* species

In culture negative PVE consider mycobacteria and moulds such as *Aspergillus* sp. as possible causes of PVE (43,44).

4.5.7 Anaerobic bacteraemia

Anaerobes are an important cause of bacteraemia and should be routinely tested (31,45).

Organisms most commonly associated with anaerobic bacteraemia include (31):

- Gram negative bacilli, including *Bacteroides* and *Fusobacterium* species
- *Peptostreptococcus* species
- *Clostridium* species

4.5.8 Immunocompromised patients

In patients who are immunocompromised, there is a high incidence of infection caused by organisms that are typically non-virulent. They form part of the normal host and environmental flora. Immunocompromised patients range from the critically ill to those with aplastic neutropaenia, where the risk of infection is greatest and in whom Gram negative bacteria cause severe sepsis associated with a high mortality rate. Even those with relative neutropaenia are at risk of sepsis (46).

Hyposplenic or asplenic patients are susceptible to fulminating sepsis caused by a variety of organisms, particularly capsulate bacteria such as *S. pneumoniae*, *H. influenzae* and *N. meningitidis*, but also less common organisms such as *Capnocytophaga* species (28,47).

The spectrum of organisms detected reflects lengthening periods of neutropaenia and duration of hospital stay, increased use of indwelling central venous catheters (CVC) and of broad-spectrum antibiotics. Polymicrobial infections are more common in this group of patients and the number of Gram positive and opportunistic infections, particularly those caused by fungi and *Mycobacterium* species, has also increased (47).

In addition to the organisms associated with bloodstream infection in the immunocompetent, isolates include (47):

- Non fermentative Gram negative rods, particularly *P. aeruginosa* (48)
- *Listeria monocytogenes*
- *Corynebacterium* species
- *Candida* and associated ascomycetous yeasts and other fungi
- Coagulase-negative staphylococci
- Viridans streptococci
- Many viruses have the potential to cause sepsis in an individual who is immunocompromised.

Other organisms including a variety of bacteria and fungi may be isolated, many of which have very specific growth requirements (49,50).

4.5.9 Fungaemia and deep seated or disseminated fungal infections

Systemic fungal infections are typically caused by opportunistic fungal pathogens in immunocompromised hosts. However, invasive disease can occur in immunocompetent individuals if the exposure is high (e.g. trauma with contaminated material) or with primary (dimorphic) fungal pathogens (causes of endemic mycoses including Coccidioides and *Talaromyces* sp.). Many systemic fungal infections originate either in the lungs (*Aspergillus, Cryptococcus, Mucorales,* as a result of inhalation) or from endogenous flora (Candida and associated ascomycetous yeasts as a result of infected lines or leakage from the gastrointestinal tract) and can spread to other organs.

Candidaemia, aspergillosis, pneumocystosis and cryptococcosis are the most common invasive fungal infections in the UK. *Candida* grows in standard blood culture as does *Cryptococcus* although the latter is rare (CSF is a more sensitive sample type). However, the sensitivity of blood culture for these yeasts is generally low and higher volume of blood needs to be collected. *Pneumocystis jirovecii* infections cannot be detected by blood culture. Aspergillus species is extremely rare whilst some moulds (e.g. *Fusarium*) can be detected in blood culture. Endemic (dimorphic) fungi can be detected by blood culture, typically presenting in yeast form. Overall, fungal infections are a diagnostic challenge, and a combination of investigations is often required to confirm the diagnosis.

Systemic fungal infections are medical emergencies and have high mortality rates, especially if appropriate therapy is delayed. Therefore, antifungal treatment is often initiated when infection is suspected clinically, and diagnostic tests should be used as part of antifungal stewardship to guide the cessation of unnecessary therapy. Antifungal resistance is an

emerging problem, and all isolates should be identified and tested for their susceptibility profile.

Microscopy and culture of normally sterile body fluids or samples such as BAL from these sites is a rapid way of detecting invasive fungal infections and helpful in interpreting the results of molecular and biomarker tests. Histopathology provides definitive evidence of infection.

4.5.10 Viral sepsis

Many viruses have the potential to cause viral sepsis in a susceptible human population. For example, herpes simplex virus (HSV) and enteroviruses are the most common viral causes of neonatal sepsis, whilst enteroviruses and human parechoviruses commonly attribute to viral sepsis in young children.

There is awareness of rare but fatal presentations such as primary HSV in peripartum and early post-partum period presenting as sepsis with multi-organ failure without typical course of vesicular lesions from recent cases of 2 maternal deaths that has prompted discussion regarding review of national Royal College of Obstetricians and Gynaecologists (RCOG) guidance where viral infections must routinely be considered as a possible cause of postpartum infection (51).

Many viruses have the ability to cause sepsis in the immunosuppressed individual, for example, hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients are at high risk of infection (primary infection or reactivation) with cytomegalovirus (CMV) and other herpesviruses. Viral reactivation is a prominent feature of herpesvirus infections as these viruses have the ability to form latent infections. Immunosuppressed individuals will often be monitored or receive prophylactic therapy targeting these viruses. In addition, respiratory viruses including respiratory syncytial virus (RSV), influenza, parainfluenza and adenovirus are cause for concern in these patient groups.

Viruses identified may be the single causative agent of sepsis (for example dengue, the most common cause of viral sepsis in tropical countries) or the virus may predispose to secondary bacterial sepsis such as that seen with influenza and staphylococcal sepsis. Other viruses may be coinfections the significance of which is difficult to determine emphasising the need for interpretation with clinical context. Diagnosis of viral sepsis is useful to inform treatment where antiviral therapy is available and appropriate.

SARS-CoV-2 is also a causative agent of sepsis. The effects on the respiratory system are well-known and in the pre-vaccination era, most people requiring hospital admission developing pneumonia of varying severity; however, virtually all other organ systems can be affected. This is consistent with a combination of direct viral invasion and sepsis.

Molecular detection of viruses in blood, respiratory specimens, CSF, skin swabs, faeces and other sample types may be appropriate depending on the clinical history and presentation of the patient and is the preferred methodology especially for patients who are immunocompromised and therefore may not mount a serological response to viral infection. Serological testing may be used in conjunction with molecular methods to aid investigations. Appropriate testing should be discussed with local virology teams.

4.6 Methods

4.6.1 Automated blood culture analysers

The introduction of fully automated, continuous monitoring blood culture analysers has led to earlier detection of pathogens (8).

The ideal blood culture analyser produces the maximum yield of pathogen in as short a time as possible in order to have the greatest influence on patient management.

Blood cultures should, where possible be placed on the continuous monitoring blood culture machine 24 hours a day, as soon as possible after collection and ideally within a maximum of 4 hours (52-54).

The time to detection once samples are loaded is dependent on the time required for multiplication to a significant level to occur; fastidious or non-culturable organisms may fail to grow and sensitivity may be decreased when samples are taken directly after antibiotic treatment (54,55).

Traditionally, where direct placement on a machine is not possible, blood cultures have been pre incubated in a separate incubator. An inadvertent consequence of this is that certain non-fermenting, Gram negative bacteria such as *Pseudomonas aeruginosa*, *Streptococcus* species and yeasts may not be detected (53,56). Therefore, this practice is no longer recommended. As a result, many laboratories now store and transport delayed samples at room temperature overnight (57,58).

However, if stored at room temperature prior to loading the time from collection to a positive result being flagged (time to positivity) for many organisms may be doubled or tripled and the sensitivity of the blood culture reduced (59). Therefore, these practises should not be used routinely (53).

4.6.2 Molecular and other technologies

Laboratories may consider the use of molecular tests at various stages of investigation depending on what analysers are available. Some technologies operate directly on whole blood whilst others can be applied to inoculated culture bottles. Technologies that detect the most common antibiotic resistance genes are also available (60,61). Local protocols should be developed to determine when molecular tests should be undertaken.

There are several commercially available methods that can detect organisms directly from EDTA blood samples, including a magnetic resonance detection platform. These include systems that can quickly detect molecular targets and provide rapid pathogen identification and detection of resistance factors. However, AST is still required hence blood cultures would still be needed. These methods can be considered an option for point of care testing (POCT), as an adjunct rather than a standalone method for diagnosis of bacteraemia or fungaemia.

Rapid identification of organisms from positive blood cultures is possible due to a number of commercially available platforms which utilise molecular techniques. For example, DNA microarray, nested PCR and amplicon rescue multiplex PCR. These techniques can also

identify the presence of resistance genes for example mecA, VanA and VanB, although the presence of a gene does not necessarily relate to its expression and phenotypic characterisation may still be required.

The decision to use molecular and other technologies to diagnose organisms causing sepsis should be made locally, taking into account logistics, patient need and geographic and local factors.

4.6.3 Factors affecting detection of causative organisms

A number of clinical factors may affect the isolation of the infecting organism, regardless of the system employed (8),(28). The diagnosis of infection and likelihood of positivity relies on the attending clinician. For the diagnosis of sepsis and systemic or disseminated infections, samples should be collected from all affected sites where possible to improve the sensitivity and specificity of the diagnostic work up. In addition to blood, these include biopsies, joint aspirates and other tissue samples. Please see scope of document for associated UK SMIs covering other sample types for the diagnosis of sepsis and systemic or disseminated infections.

Method of collection

Studies on blood culture sample collection have shown that discarding the first 10mL aliquot of blood taken from vascular catheters has no effect on the contamination rate of these samples and that, even following strict sterile precautions; samples taken from central venous catheters have higher contamination rates than those taken from peripheral or arterial lines (62,63). Arterial blood offers no advantage over venous blood for detection of most micro-organisms, although it has been reported as being superior in detecting disseminated fungal disease (64). Changing needles between venepuncture and inoculation of the bottles is not recommended because this carries a risk of needle stick injury. Needle changing does not reduce contamination rates according to some authorities, but slightly reduces contamination according to a meta-analysis (15),(65,66).

Contamination of blood cultures complicates interpretation and may lead to unnecessary antimicrobial therapy and increased costs. In general, contamination target rates are set at less than 3% (66,67). Several criteria are used to differentiate between contamination and true bacteraemia and to determine the clinical significance of a positive result. These include the identity of the organism, the number of positive sets, the number of positive bottles within a set, quantity of growth, and clinical and laboratory data (including source of culture) (66),(68). Prevention of contamination can be achieved through appropriate skin and bottle preparation, obtaining cultures from peripheral venepuncture instead of vascular catheters, and through training and intervention measures (68,69).

Less is known about the impact of blood collection method on molecular and biomarker test false positivity rates. When using more sensitive methods, contamination is likely to be an important issue to consider.

Volume, number and timing of samples

Blood culture volume is the most significant factor affecting the detection of organisms in bloodstream infection. There is a direct relationship between blood volume and yield, with approximately a 3% increase in yield per mL of blood cultured. False negatives may occur if inadequate blood culture volumes are submitted (70). Automated blood culture analysers may offer the facility to monitor bottle weight or to estimate volume of blood. Ideally samples are collected before starting antimicrobial therapy, but ongoing antimicrobial treatment is not a reason for not collecting blood cultures.

The number of organisms present in adult bacteraemia is frequently low, often less than 1×10^3 colony forming units per litre (cfu/L) (71). For adult patients it is recommended that 20 to 30mL of blood be cultured per set (72,73). Most modern commercial analysers allow 10mL blood to be added to each bottle. Manufacturers' optimum blood volume recommendations vary and should be followed.

Data regarding the optimum total blood volume per set for neonates and children are limited. The criteria for calculating total blood culture volume is often based on weight rather than age and relates to total patient blood volume (70). In infants and children, the magnitude of bacteraemia is usually higher than that in adults; therefore, sensitivity of detection is not significantly reduced by lower blood to medium ratio. Consideration should be given on how to feed the blood volume information back to users.

Low level bacteraemia (less than 4×10^3 cfu/L) in neonates and children does occur with clinically significant organisms. One study suggests that for the reliable detection of low level bacteraemia, 4.0 to 4.5% of a patient's total blood volume should be cultured (74).

Candidaemia is a diagnostic challenge as the concentration of *Candida* and associated ascomycetous yeasts in the blood during candidaemia is very low with up to 65% of positive blood cultures having less than 1 colony forming unit per millilitre. Therefore, when candidaemia is suspected a higher volume of blood (3 sets, 60 mL) needs to be collected to improve the sensitivity of detection. The addition of blood culture bottles containing media optimised for fungal growth (e.g. lytic media) can increase the detection of *Candida* from blood compared with standard blood culture media (75,76). Some *Candida* sp. demonstrate improved recovery in anaerobic media. The low concentration and intermittent presence of Candida in the blood stream is also a major challenge for the polymerase chain reaction (PCR).

For the majority of patients, 2 sets of blood culture bottles (2x2 bottles, 40mL) are recommended to detect bacteriaemia. These can be collected in one draw (77). A third set taken from a different site increases yield although it also increases the risk of contamination (78). However, collecting a third set (total of 3x2 bottles, 60mL) is recommended if candidaemia is suspected. If endocarditis is suspected, 3 sets of blood culture bottles (3x2 bottles, 60mL) should be collected as separate draws over a 24h period. Samples should be taken as soon as possible after a spike of fever as fevers and rigors occur 30 to 60 minutes after the entry of organisms into the bloodstream. However, 1 study has shown no significant difference in isolation rates for blood drawn either at intervals or taken simultaneously with

fever spikes (79). Timing of sample collection may be less important in cases of continuous bacteraemia.

Previous or concurrent antimicrobial therapy

Ideally, blood samples should be taken prior to initiation of antimicrobial treatment. Any recent or ongoing antimicrobial therapy can have a significant detrimental effect on the sensitivity of blood culture, which may result in false negative results. On the other hand, samples collected whilst the patient is on treatment can reveal a new resistant causative pathogen.

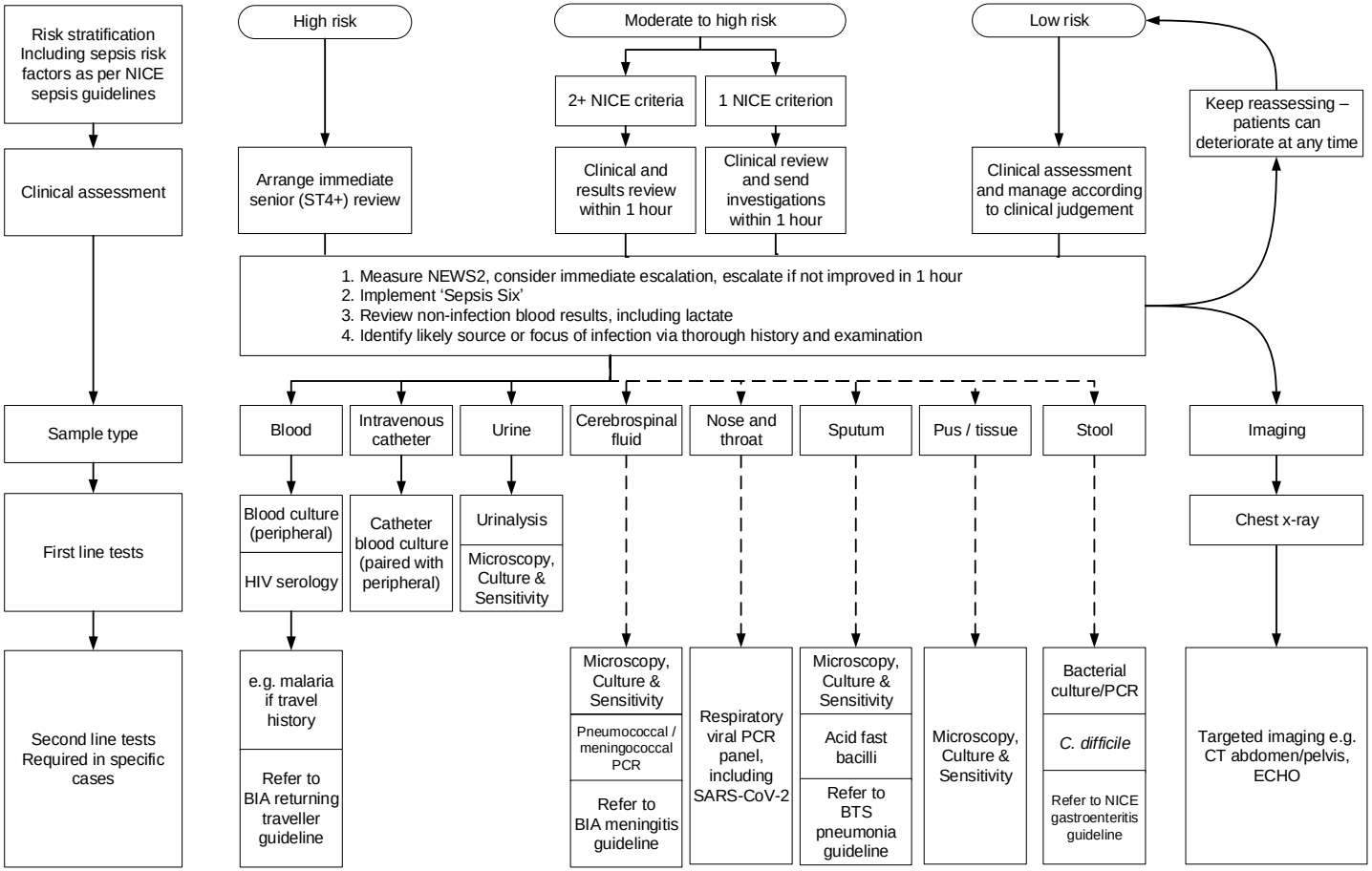
Media containing antibiotic inactivating resins and other adsorptive materials including charcoal have been developed to overcome the effect of antimicrobials (80). Some media, however, rely on optimal blood broth dilution for antimicrobial neutralisation (81).

4.6.4 Post-mortem blood cultures

Post-mortem blood cultures have been shown to be associated with significantly higher positive rates than blood cultures sampled during life. Results of post-mortem blood cultures and their clinical significance should be interpreted with caution. However, they may be useful in the investigation of sudden unexpected death in infants and children (SUDI) (81-85).

4.7 Algorithm 1: Investigation of sepsis in adult hospital patients

The flowchart is intended as a general resource for acute medicine and infection specialists involved in the management of adults with suspected sepsis. It should be used in conjunction with current UK guidance on the management of sepsis, which it does not replace. This flowchart is not for use in sepsis in the immunocompromised host including neutropenic sepsis – see NICE neutropenic sepsis guidance. The flowchart was developed in association with the British Infection Association where the full coloured poster version can be found.



Notes to algorithm 1

The Sepsis Six

The ‘Sepsis Six’ are the following:

- Ensure senior clinician attends (ST4+)
- Give oxygen if required
- Obtain IV access, take bloods
- Give antibiotics
- Give IV fluids
- Monitor (including urine output, NEWS2, lactate)

National Early Warning Score (NEWS) 2

A chart describing the NEWS2 scoring system is available on the Royal College of Physicians website.

In the case of infection in combination with a NEWS2 score of 5 or greater, THINK SEPSIS – assess urgently and consider escalation to critical care.

Risk factors for sepsis

Refer to the risk stratification tools provided in NICE guideline NG51 Sepsis: recognition, diagnosis and early management. Risk factors include the following:

- Extremes of age (less than 1 year or greater than 75 years) or frailty
- Recent trauma, surgery or invasive procedure
- Impaired immunity
- Indwelling devices, intravenous drug misuse, any breach of skin integrity
- Note additional risk factors in pregnancy

Antimicrobial considerations

Remember – start smart, then focus. Refer to the antimicrobial stewardship toolkit.

Broad spectrum antimicrobials as per local sepsis guidance should be given within 1 hour. If the patient is immunocompromised, has a history of previous antimicrobial resistance or other complicating factors, antimicrobials should be discussed with the microbiology department.

Review antimicrobials within 48 hours or when microbiological results are available.

In the absence of a confirmed microbiological diagnosis, consider the need for antimicrobials.

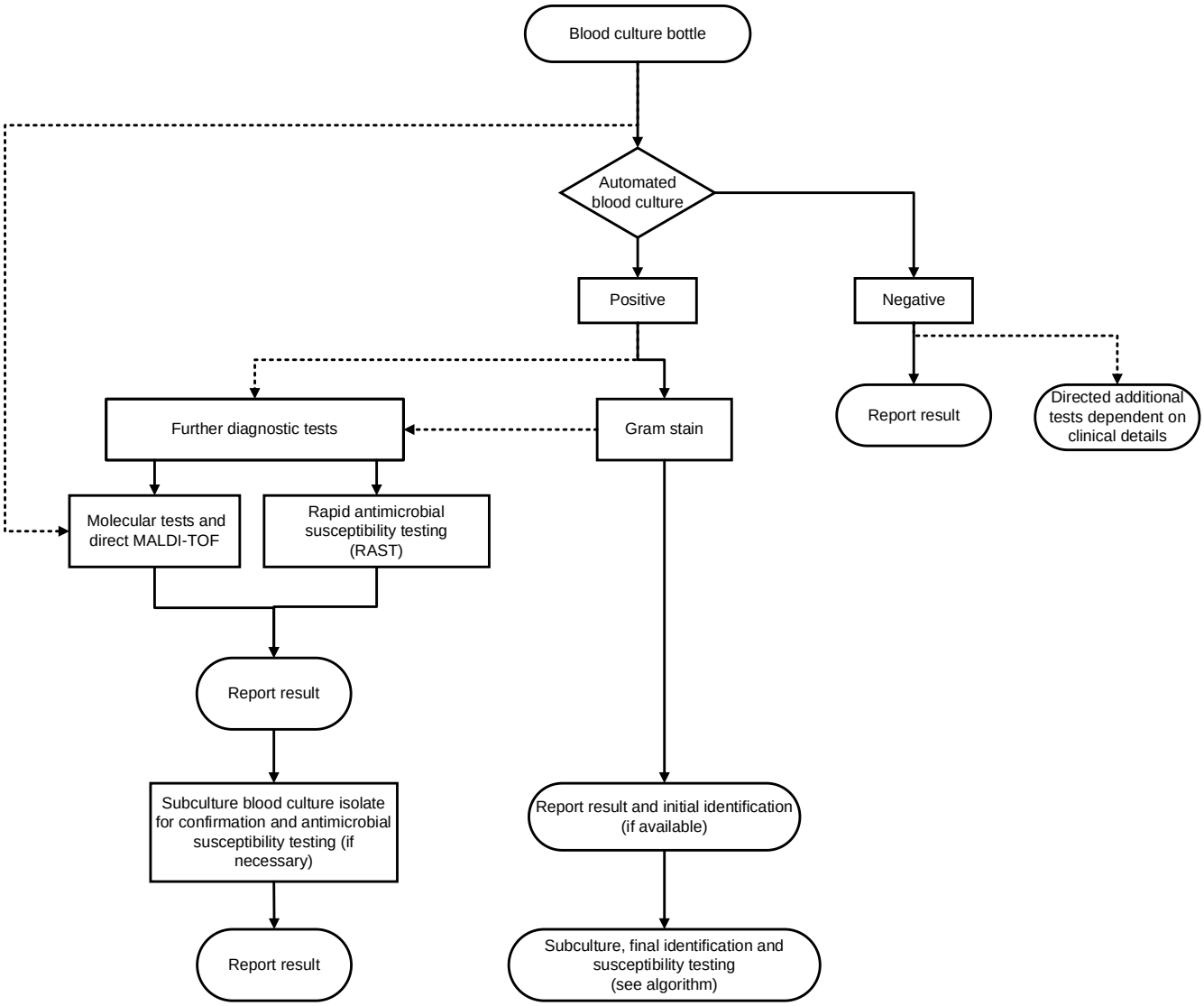
Additional considerations

Blood culture should always be performed in suspected sepsis. Refer to section 5.3 on adequate quantity and appropriate number of specimens. If there is a clear source of infection, cultures of other sites than the likely source of the infection are generally not needed apart from blood culture.

If infection such as intra-abdominal, pelvic, joint or necrotising fasciitis is suspected, refer early; prompt surgical and radiological management is essential.

If line infection is suspected, consider line removal.

4.8 Algorithm 2: Investigation of blood culture bottles



Notes to algorithm 2

- Laboratories may consider the use of molecular tests at several stages depending on what analyser is available. Analysers may be available that operate directly on inoculated culture bottles, either before incubation or following a positive flag. Local protocols should be developed to determine when molecular tests should be undertaken.
- If fungi are seen on microscopy, specialised stains or molecular tests (PCR, pan-fungal sequencing, PNA-FISH) may be required
- Blood culture is the standard test for microbial diagnosis of endocarditis; however, directed serological testing may be considered in culture negative cases (Q fever

serology) (86). Serological testing may also be helpful if exposures are suggestive of *Brucella* endocarditis

- Testing serum for Beta-D-glucan (BDG) may be used to rule out candidaemia; false positives are common hence results should be interpreted in the wider clinical context including additional mycological evidence.
- Reasons for negative blood cultures include concomitant or antecedent antimicrobial therapy, or presence of fastidious organism that do not grow in routine blood culture (however, the latter is less frequent).
- Detection of viral pathogens in certain clinical scenarios particularly in immunocompromised patients. An EDTA plasma/whole blood may be required for virology testing.

5 Pre laboratory processes (pre analytical stage)

5.1 Specimen type

The types of specimens include:

- Blood for
    - Blood culture
    - Molecular tests for viruses (PCR), bacteria (such as TB PCR and 16S sequencing) and fungi (such as *Aspergillus* and *Pneumocystis jirovecii* PCR and pan-fungal sequencing), as clinically appropriate
- Serum or plasma for
    - Fungal biomarker and cryptococcal antigen tests
    - Viral serology
- CSF for
    - Bacterial and fungal culture (see UK SMI B 26 – Investigation of fluids from normally sterile sites)
    - Molecular tests for viruses (PCR), bacteria (such as TB PCR and 16S sequencing) and fungi (such as *Aspergillus* and *Candida* PCR or pan-fungal sequencing).
    - Fungal biomarker and cryptococcal antigen tests
- Urine for
    - Urinalysis and culture
    - Bacterial antigen tests (pneumococcus, legionella)
- BAL and respiratory samples for
    - Bacterial and fungal culture
    - Molecular tests for respiratory viruses (PCR), CMV/HSV/VZV, bacteria (such as TB PCR and 16S sequencing) and fungi (such as *Aspergillus, Mucorales* and *Pneumocystis jirovecii* PCR, pan-fungal sequencing).
    - Fungal biomarker tests (excluding 1-3-β-D-glucan)
- Blister swabs, skin biopsies and other tissue for
    - Molecular tests for viruses, fungi (such as *Aspergillus, Mucorales* PCR), 16S and pan-fungal sequencing (as appropriate)
    - Bacterial and fungal culture. Other specimens may be processed in blood culture bottles where appropriate (see UK SMI B 26 – Investigation of fluids from normally sterile sites and UK SMI B 38 – Investigation of bone marrow).

5.2 Specimen collection, transport and storage

Please see the *Best Practice Standards for the delivery of NHS infection services in the UK* for maximum times for processing and availability of results for time critical samples (from the time of collection) (87).

General

Collection of blood from the patient should be carried out following Department of Health and Social Care guidance (67,88,89).

Compliance with postal, transport and storage regulations are essential.

For safety considerations refer to Section 2: scientific information at the top of the document.

Use aseptic technique.

Blood culture

Collect specimens in appropriate CE or UKCA marked leak proof containers (according to manufacturers' instruction if using a continuous monitoring blood culture analyser) and transport in sealed plastic bags. Appropriate blood culture bottles must be used for specific machines when using continuous monitoring blood culture analysers and manufacturers' instructions should be followed. Consider the use of a single low volume bottle for small volumes of blood. If a low volume bottle is unavailable, use a single aerobic bottle.

Inspect the blood culture bottles for damage and ensure that the blood culture bottles have not exceeded their expiry date.

Collect specimens before antimicrobial therapy where possible (13,89).

Collect specimens as soon as possible after the onset of clinical symptoms. Although blood can be sampled at any time, drawing blood at, or as soon as possible after a fever spike is optimal (13), except in endocarditis where timing is less important (79).

Note: The use of iodine based disinfectants is not recommended for disinfection of the butyl rubber septum for some commercial systems as this may affect the integrity of the septum. Follow local guidance (90).

Note: The use of blood collection adapters without 'winged' blood collection sets is not recommended as it is not possible to accurately judge the sample volume and there may be the potential for backflow of blood culture media to patient veins.

Note: If blood for other tests such as blood gases is to be taken at the same venepuncture, the blood culture bottles should be inoculated first to avoid contamination. It is preferable to take blood for culture separately.

Culture of other sample types

For collection, transport and storage of other samples including samples from sterile sites and viruses, and for maximum times for processing and availability of results for time critical

samples (from the time of collection), please see the *Best Practice standards for the delivery of NHS infection services in the UK* (87).

Molecular tests

Follow manufacturers' instructions and local testing laboratory user manual for appropriate transportation tubes and pots for sample collection (87).

Naïve samples stored for prolonged periods at ambient (non-refrigerated/frozen) conditions may be prone to nucleotide degradation.

Biomarker tests and serology

Follow manufacturers' instructions and local testing laboratory user manual for appropriate for sample collection.

Please note that the BDG test should be taken into a separate tube and only processed at the laboratory performing the test in BDG free environment and equipment to avoid false positive results (87).

5.3 Adequate quantity and appropriate number of specimens

Blood culture

A blood culture set is defined as one aerobic and one anaerobic bottle (up to 10mL of blood per bottle). Some proprietary media collection systems may be presented as a single bottle; manufacturer's guidance on volume should be followed (91).

For infants and neonates, specific paediatric blood culture bottles are available, please follow manufacturer's instructions.

Quantity

Adults

Preferably, a total volume of at least 40mL (two sets) should be collected (92). In case of suspected endocarditis and fever of unknown origin, an extra set is required to improve the sensitivity of the test (a total volume of up to 60 mL).

Candidaemia is a diagnostic challenge as the concentration of *Candida* and associated ascomycetous yeasts in the blood during candidaemia is very low. 60 mL (3 sets of 2 × 10 mL samples) of blood must be collected within a 30 minute period to achieve sensitivity of 50–75% (93). The use of lytic media can improve the recovery of *Candida* sp.

Children and neonates

No more than 1% of the total blood volume (88).

Note: Do not exceed the manufacturer's recommended maximum volume for each bottle. They are specifically designed to maintain the usual blood to broth ratio (1:5 to 1:10).

Note: If the volume of blood is insufficient for 2 bottles, the aerobic bottle should be inoculated first and then the rest inoculated to an anaerobic bottle (89).

Number

The number and frequency of specimen collection is dependent on the clinical condition of the patient.

Take 2 consecutive sets from 2 separate venepuncture sites during any 24 hour period for each septic episode. For neonates, take a single aerobic bottle or special low volume bottle (88).

If a central line is present, blood may be taken from this and from a separate peripheral site when investigating potential infection related to the central line; the peripheral vein sample should be collected first (67,89).

Take 2 sets during the first hour in cases of sepsis prior to commencing antibiotic treatment, provided this does not significantly delay antibiotic administration (17).

Take at least 3 sets during a 24 hour period where the patient has suspected infective endocarditis (93).

Culture of other sample types

Number and timing of tissue samples

Ideally, specimens should be taken prior to antimicrobial administration. If a patient is already on antimicrobials, consideration should be given as to whether it is safe to stop antimicrobials prior to sampling. The patient's condition will determine if this is possible.

The specimen should ideally be large enough to carry out all microscopy preparations and cultures. Minimum specimen size will depend on the number of investigations requested. Number and frequency of specimen collection are dependent on clinical condition of patient. For prosthetic joint specimens, 5 or more samples may need to be taken at the time of surgery to determine if the organism detected on culture is the true pathogen for the infection.

Molecular tests

Follow manufacturers' instructions and local testing laboratory user manual for appropriate sample volume.

Biomarker tests and serology

Follow manufacturers' instructions and local testing laboratory user manual for appropriate for appropriate sample volume.

5.4 Optimal transport and storage conditions

Blood culture

Specimens should be transported and processed as soon as possible (94-97).

Inoculated bottles should be loaded onto automated blood culture analyser (or incubated in the case of non-automated analysers) without delay and ideally within a maximum of 4 hours from time of collection (92).

Samples should not be refrigerated or placed in a pre incubator (91).

Samples should be retained in accordance with The Royal College of Pathologists guidelines ‘The retention and storage of pathological records and specimens’ (98).

It is recommended that laboratory management establish and manage transportation of samples to ensure specimens arrive within an appropriate time frame dependent on specimen type and tests required, and to prevent sample deterioration (99).

Laboratory workers should be aware that delayed sample bottles should be checked for signs of growth prior to loading. If signs of growth are visible a Gram stain should be performed and the bottle subcultured (100).

Molecular tests

Follow manufacturers’ instructions and local testing laboratory user manual for acceptable transportation time and appropriate storage time and conditions. If samples cannot be processed on the same day, they must be kept chilled or frozen until processed.

Biomarker tests and serology

Follow manufacturers’ instructions and local testing laboratory user manual for acceptable transportation time and appropriate storage time and conditions.

5.5 Specific safety considerations

Blood culture

All specimens should be processed at Containment Level 2 unless infection with a Hazard Group 3 organism (for example *Mycobacterium tuberculosis*, *Brucella* species, *Francisella* species, *Y. pestis*, *B. mallei*, *B. pseudomallei* or endemic fungi) is suspected. In these situations, work should be performed in a microbiological safety cabinet under Containment Level 3 conditions.

Laboratory procedures that give rise to infectious aerosols (including venting of blood culture bottles) should be conducted in a microbiological safety cabinet (MSC). Ideally all blood cultures should be subcultured in an MSC because clinical details may be lacking and may not highlight the possibility of Hazard Group 3 organisms.

N. meningitidis is a Hazard Group 2 organism and the processing of diagnostic samples can be carried out at Containment Level 2. However, *N. meningitidis* causes severe and sometimes fatal disease. Laboratory acquired infections have been reported. Due to the severity of the disease and the risks associated with generating aerosols of the organism, any manipulation of suspected isolates of N. meningitidis should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols).

Be aware that some of the Hazard Group 3 fungi are thermally dimorphic and will typically grow as yeast forms in blood culture bottles and subcultures at 37°C, whereas they grow as the highly infective mould form when subcultured to agar incubated at 28 to 30°C. Care should be taken with yeast isolates if there is a relevant travel history (e.g. USA, South and

Central America, Africa and South East Asia), especially in the immunocompromised patients.

The use of sharp objects should be avoided wherever possible. The use of airway needles for venting and sub vent units for the subculture of bottles are preferred, unless the system uses a screw cap in which case the use of a plastic pipette is recommended.

Load bottles from ‘high risk’ patients according to manufacturers’ recommendations and local protocols.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI (94-96,98,101-119).

The above guidance should be supplemented with local COSHH and risk assessments.

Molecular tests and Biomarker tests and serology

Follow manufacturers’ instructions for appropriate safety considerations.

6 Laboratory processes (analytical stage)

Blood Culture bottles

6.1 Specimen processing

6.1.1 Appearance

Visually inspect the contents of bottles for evidence of microbial growth (such as haemolysis, gas production or turbidity) prior to loading on to automated analysers.

6.1.2 Sample preparation

N/A

6.1.3 Specimen processing

Incubate the bottles at 35 to 37°C for 5 to 7 days (see section 6.3.1). Loading may be on to a remote incubation device.

Standard

Positive bottles from all analysers

Disinfect the septum of the blood culture bottle with the appropriate disinfectant and allow to dry.
Withdraw a few drops of blood broth mixture (or buffy coat layer) with a sub vent unit or plastic pipette, depending on bottle type, and inoculate 1 drop on to each agar plate (see section 6.3.1).
For the isolation of individual colonies, spread inoculum with a sterile loop (UK SMI Q 5 – Inoculation of culture media for bacteriology).

Subculture for direct susceptibility testing. If the correct inoculum is not achieved the test should be repeated.
Note: In order to minimise the risk of autolysis of certain organisms such as *S. pneumoniae*, bottles should be sub cultured as soon as possible after a positive flag is detected (120).

Positive bottles from manual systems

Subculture all bottles of the set as described above, even if only 1 bottle appears positive.

Negative bottles from manual systems

Perform blind subculture for any aerobic bottle that appears negative after 24 to 48 hours (121) .

Supplementary

Flag or appearance positive, but culture negative for all automated analysers

Examine the growth curve.
If possible, exclude the possibility of false positives due to high white cell counts.
In relation to the clinical presentation and Gram stained film result, consider the possibility of a nutritionally dependent, slow growing or fastidious organism. Subculture to appropriate media or if uncertain as to possible aetiology, perform supplementary culture as indicated in Section 6.3.1. Refer to section 6.6 Specific technical limitations for further information.

Manual systems or automated analyser failure

If using a manual system, or in the case of automated analyser failure, bottles should be inspected at intervals (for example, day 1, day 2 and day 7).

6.2 Microscopy

Positive bottles for all analysers

Perform microscopy on broth from any bottle which ‘flags’ positive or which is visually positive (bowed septum, blood lysed or indicator colour change).

1. Mix the bottle gently by inversion if this has not already been done automatically.

   Note: Some analysers may not require mixing, but manufacturers may recommend subculture of the buffy coat layer.

2. Disinfect the septum of the blood culture bottle with the appropriate disinfectant and allow to dry.

3. With a sub vent unit or plastic pipette, depending on bottle type, remove a few drops of blood broth mixture (or buffy coat layer) and place on a clean microscope slide.

   Note: Refer to manufacturers’ instructions with respect to preparing smears from charcoal containing bottles.

4. Spread with a sterile loop to make a thin smear for Gram staining.

   Note: Gram negative organisms may be seen more easily if Sandiford or carbol fuchsin counterstain is used (122) (UK SMI TP 39 – Staining procedures).

If organisms are not seen on microscopy:

1. Investigate the growth curve (automated analysers). If growth parameters indicate positive microbial growth, the preparation of further films with alternative stains may be useful.
2. Subculture to agar plates (see 4.5.1), and return the bottle to the automated analyser, according to manufacturer's instructions, for further incubation and testing.
3. Consider *Mycobacterium* species. UK SMI B 40 – Investigation of specimens for Mycobacterium species.

On automated analysers false positive signals may be caused by excess blood volume or a high white cell count. Manufacturer’s recommendations should be followed.

6.3 Culture and investigation

Inoculate each agar plate using a sterile pipette (UK SMI Q 5 – Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

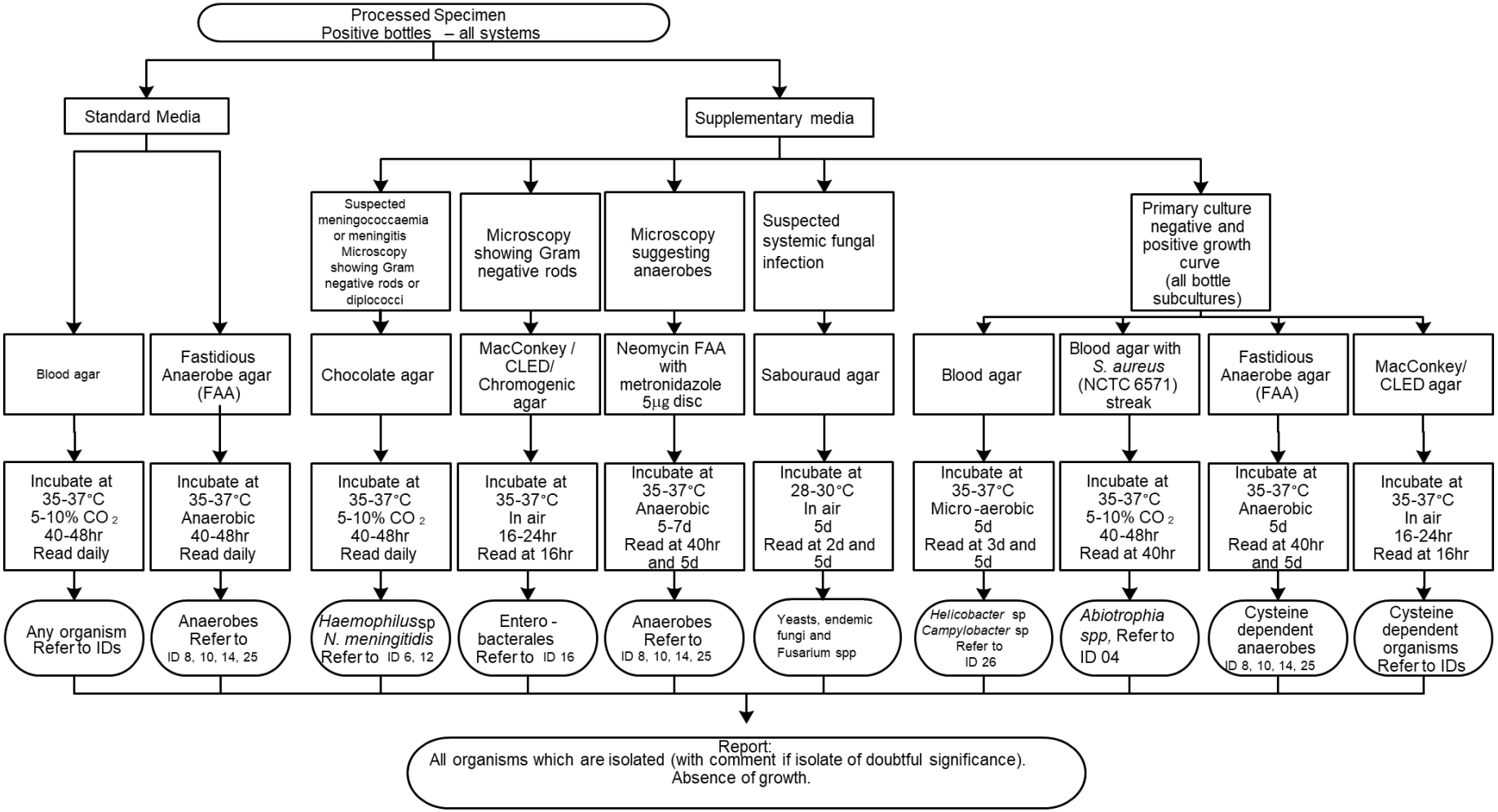
6.3.1 Table 1: Culture media, conditions and organisms

Clinical details or conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
All clinical conditions	Blood	Blood agar†	35 to 37	5 to 10% CO_2	40 to 48hr*	Daily	Any organism may be significant
		Fastidious anaerobe agar	35 to 37	anaerobic	40 to 48hr*	greater than or equal to 40hr and up to 5d	Any organism may be significant
For these situations, consider adding the following:							
Clinical details or conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Suspected meningo-coccaemia or meningitis Small Gram negative rods or diplococci seen on microscopy	Blood	Chocolate agar†	35 to 37	5 to 10% CO_2	40 to 48hr	Daily	*Haemophilus* species *N. meningitidis* *N. gonorrhoeae*
Gram negative rods seen on microscopy	Blood	MacConkey, CLED, or Chromogenic agar	35 to 37	air	16 to 24hr	greater than or equal to 16hr	Enterobacterales Non–fermentative organism *Pseudomonas* species
Microscopy suggestive of mixed or anaerobic infection	Blood	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35 to 37	anaerobic	5 to 7d	greater than or equal to 40hr and at 5d	Anaerobes

Suspected systemic fungal infection§#	Blood	Sabouraud agar	28 to 30	air	5d#	2d and at 5d	Yeasts, endemic fungi and *Fusarium* species
Primary culture negative and positive growth curve‡ (subculture all bottles)	Blood	Blood agar	35 to 37	micro aerobic	5d	greater than or equal to 3d and at 5d	*Campylobacter* species *Helicobacter* species
		Blood agar with streak of *S. aureus* (NCTC 6571)	35 to 37	5 to 10% CO_2	40 to 48hr	greater than or equal to 40hr	*Abiotrophia* species
		Fastidious anaerobe agar	35 to 37	anaerobic	5d	greater than or equal to 40hr and at 5d	Cysteine dependent anaerobic organisms
		MacConkey or CLED agar	35 to 37	air	16 to 24hr	greater than or equal to 16hr	Cysteine dependent organisms
Other organisms for consideration – *Mycobacterium* (B 40) and *Brucella* species: also consider organisms that might be involved in deliberate release.							
† an optochin disc may be added if streptococci seen on microscopy. * Incubation may be extended to up to 5 days if false negative likely or as clinically indicated; in such cases plates should be read at greater than or equal to 40 hours and left in the incubator or cabinet for up to 5 days. § If yeasts are seen on microscopy: add chromogenic agar, incubate at 35-37°C for up to 48hrs, check plates daily # where clinically indicated, blood culture bottles may require an extended incubation of up to 3 weeks for *Cryptococcus* species and up to 6 weeks for *Histoplasma* species (123),(124),(125),(126). ‡other organisms may need to be considered.							

Rapid tests such as antigen detection or NAATs should be performed according to manufacturers' instructions.

6.3.2 Algorithm 3: Culture media, conditions and organisms for investigation of blood cultures (for organisms other than Mycobacterium species)



6.4 Identification

Refer to individual UK SMIs for organism identification.

All clinically significant isolates should be identified to species level.

Note: Any organism considered to be a contaminant may not require identification to species level.

Organisms may be identified further if clinically or epidemiologically indicated. Any organism isolated from a normally sterile site should be identified to species level.

It is recommended that clinically significant isolates are retained for at least 1 week. Storage of isolates on slopes of appropriate media or at -20°C to -80°C for longer periods may need to be considered if further testing is likely (for example typing isolates from nosocomial infection).

The use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry for identification of positive cultures from most clinical specimens and sites has greatly reduced the time from isolation of a positive culture to its identification and antimicrobial susceptibility testing. MALDI-TOF can also be used to directly identify organisms from a positive blood culture broth. The MALDI-TOF procedure for the identification of filamentous fungi is not yet optimized and MALDI-TOF databases may only contain limited fungal species.

Please see UK SMI TP 40 – MALDI TOF MS test procedure.

Use of polymerase chain reaction (PCR) tests and other nucleic acid amplification tests (NAATs) for identification of bacteria from positive blood samples can enable the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short time frame, particularly if multiplex real time PCR is used (127). Several assays are available including pathogen specific assays, broad range assays and multiplex assays.

6.5 Antimicrobial susceptibility testing

To reduce turnaround times, it is recommended a standardised and verified direct susceptibility test such as the EUCAST Rapid Antimicrobial Susceptibility Test (RAST), should be performed on all positive blood culture isolates where possible. In house direct testing methods on all positive blood cultures may also be used, but will require local, documented validation. It should be recognised that sometimes different organisms may be identified from different bottles within a pair.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) RAST method (128) has been shown to provide reliable antimicrobial susceptibility testing results for relevant antimicrobial agents and bloodstream infection pathogens after 4 to 6 hours of incubation. For further information refer to EUCAST guidelines. Laboratories should validate or verify all methods used, as appropriate.

Antifungal testing should be undertaken on all blood culture isolates following recommendations from EUCAST. If this is not possible in house then isolates should be referred.

Many technologies can be used to provide rapid and/or automated/semi-automated antimicrobial susceptibility testing (AST). These include nucleic acid amplification, whole genome sequencing, and hybridization as well as immunodiagnostic and mass spectrometry-based methods and biosensor-based AST. Both AST and rapid identification of organisms are essential in the diagnosis of the pathogens causing sepsis in patients. Knowledge of the antibiogram allows for initiation of appropriate antimicrobial therapy which could help reduce the mortality and morbidity associated with sepsis. Rapid commercial systems for antimicrobial susceptibility testing are currently only validated for use on positive blood culture broths. However, automated and semi-automated AST systems can help to improve turnaround times as well as accuracy of results by utilising microbroth dilution or similar techniques. These reduce potential reading errors as can be seen with the traditional disk diffusion methods.

There are molecular methods for detection of mutations associated with antimicrobial resistance. Laboratories should follow manufacturer's instructions and validate or verify all methods used, as appropriate.

6.6 Specific technical limitations

6.6.1 Terminal subculture

Terminal subculture is not normally required in automated analysers. If manual or semi-automated analysers are used, subculture of both bottles in a set where only 1 bottle flags positive reveals both to be positive in about 50% of cases.

6.6.2 Intravenous catheter related infection

Differential time to positivity and differential quantitative culture as a means of diagnosing catheter related organism is not recommended by this UK SMI. This is due to issues with reliability, and its lack of applicability to cases of polymicrobial infection (129,130).

6.6.3 Inconsistent results

Positive appearance or flag positive with positive Gram stained film, but negative subculture

This may occur with *Abiotrophia* species (nutritionally variant streptococci), *S. pneumoniae* which have undergone a degree of autolysis, and fastidious organisms which are unable to grow on routine solid culture media. Additional or supplemented media, prolonged incubation or alternative growth atmosphere should be considered, depending on the microscopy and clinical indications. Organisms may include:

- *Campylobacter* species
- *Helicobacter* species
- Capnophilic organisms

- Slow growing anaerobes

Some media are reported to reduce the autolysis of *S. pneumoniae* (120). If *S. pneumoniae* is suspected, either by microscopy or clinically, it may be useful to inoculate some of the lysed blood broth mixture to fresh blood culture bottles in an attempt to recover viable organisms.

Positive appearance or flag positive with negative Gram stained film, and negative subculture

It is important to examine the growth curve on automated analysers to exclude the possibility of a false negative culture before assuming a false positive flag.

Reasons for false positivity are often multifactorial. On automated analysers they may include problems with equipment, threshold values set too low, exceeding the maximum recommended blood volume, or testing blood with high leucocyte counts. On conventional analysers, turbidity may be related to the appearance of the patient's serum rather than microbial growth. However, if growth curves indicate microbial growth, then an alternative stain such as carbol fuchsin (131), Giemsa or Sandiford may be required to demonstrate the presence and morphology of the organisms involved (122). This may give guidance for the selection of appropriate media for subcultures.

Negative appearance or negative flag with positive Gram stained film and positive subculture

Refer to section 6.1.3 under “Negative bottles from manual systems”.

6.6.4 Media used

Most systems employ different media for the isolation of aerobic and anaerobic organisms. Some media are specifically designed for the detection of organisms such as fungi and *Mycobacterium* species. A variety of blood culture media and systems have been evaluated and are commercially available. Media differ in the type and proportion of various supplements and anticoagulants, volume of broth, headspace atmosphere and the presence of antimicrobial neutralising agents. Aerobic bottles now rarely require venting when using fully automated continuous monitoring analysers (132),(133). Aerobic bottles using other systems may require transient venting to increase the oxygen content in the headspace for strictly aerobic organisms such as *P. aeruginosa* and *Candida albicans* (100).

6.6.5 Incubation time and temperature

Incubation temperature of 35 to 37°C for 5 to 7 days is recommended for routine blood cultures (8,13). The microscopic appearance of endemic fungi may differ according to incubation temperature. Five days is usually sufficient incubation time for the recovery of most organisms if automated analysers are used (13,131). If a shorter incubation duration is used, such as 4 days, laboratories must validate their processes. If conditions such as brucellosis are suspected, 2 to 5 days incubation is usually sufficient. However, the incubation period may be extended to 10 days depending on culture medium used, and a terminal subculture may be required. It is advisable that if these bacteria are suspected that all culture is suspended and the samples processed in a facility that has suitable biosecurity containment facilities.

The incubation time may be extended for some cases of suspected endocarditis, for patients on antimicrobial therapy, or when infection with fungi (such as dimorphic fungi) or unusual, fastidious or slow growing organisms is suspected (131). The increased yield may be small for some organisms (HACEK) and specialised methods rather than extending incubation times may be more likely to improve recovery (13).

7 Post laboratory processes (post analytical stage)

7.1 Reporting microscopy

Gram stain

Report organism detected.

Other supplementary stains

Organisms that are detected should be reported verbally where significant (in addition, written reports may be required by local protocols).

Microscopy reporting time

Positive results should be released immediately, ideally within a 2-hour period, following local policy, recognising that many preliminary results require specialist interpretation. Written or computer-generated reports should follow preliminary or verbal reports within 24 hours.

In certain settings, it may be safer to defer issue of results that become available during times of restricted ward and clinical microbiologist availability, and this should be decided at a local level.

7.2 Reporting culture

The following results should be reported:

- all organisms which are isolated (with comment if isolate is of doubtful significance)
- absence of growth
- results of supplementary investigations

Culture reporting time

Preliminary positive culture reports should be communicated promptly to the clinical team, for example by telephone or electronically, stating if appropriate that a further report will be issued. Final written or computer-generated reports should follow preliminary or verbal reports on the same day as confirmation where possible, and within a maximum of 24 hours (4).

Preliminary negative results should be reported at 36 hours from incubation for neonates and 48 hours for all other patients (or as per local agreement) (54,88).It is anticipated that

preliminary negative reports will be generated automatically to closely reflect the true incubation time. Final reports should be generated within 5 days of incubation in the laboratory (greater if extended incubation required, or if isolates are sent to a reference laboratory for confirmation), as soon as possible and within a maximum of 48 hours after the preliminary report.

Clinically urgent results should be telephoned or sent electronically according to local protocols.

7.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended. If molecular methods are used to detect mutations associated with resistance this should be stated in the report.

7.4 Reporting molecular results

Results obtained from molecular methods should be reported promptly in accordance with the format as described by the manufacturer's instructions or as locally validated.

7.5 Biomarker tests and serology

Detection of fungal antigens such as β-1,3-D-glucan (BDG) in serum or sterile body fluids is a sensitive (approximately 80%) but non-specific indicator of fungaemia. BDG is present on in the cell wall of most fungal pathogens with the exception of *Mucorales, Rhizopus, Blastomyces* and some other rare species and it is thus commonly called a pan fungal biomarker. Cryptocooccus species also have BDG in their cell wall but is not released into the blood stream through its capsule. Among high-risk patients, a positive BDG is strongly associated with invasive candidosis, pneumocystosis or aspergillosis although false positive results occur. Conversely, failure to detect BDG has a high negative predictive value and is used in antifungal stewardship to guide cessation of therapy. Commercial kits for detecting *Candida* specific antigens are also available. Measurement of galactomannan in serum, BAL or CSF is the most commonly used test to diagnose invasive aspergillosis. It is important to note that galactomannan is also present on some other filamentous moulds such as *Penicillium* species.

8 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see user manuals and request forms

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

All Lancefield group A, group B, group C and group G beta haemolytic Streptococci from blood cultures should be sent to the reference laboratory for free of charge typing and surveillance.

Contact the appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

- England
- Wales
- Scotland
- Northern Ireland

Unusual organisms likely to be involved in a deliberate or accidental release of infection (bioterrorism or biological warfare)

In the absence of any other risk factor (for example foreign travel, clinical laboratory or veterinary work posing an infection hazard) cases or clusters of certain organisms could suggest the possibility of a deliberate or accidental release. Such events require a rapid response; suspicion of deliberate or accidental release of micro-organisms must be notified urgently. The organisms are reportable to UKHSA under The Health Protection (Notification) Regulations 2010.

Other arrangements exist in Scotland (117), Wales (118) and Northern Ireland (114).

9 Auditable outcome measures

1. Audit of the total TAT from specimen collection to clinical action and analysis of delays in the whole blood culture specimen journey. Identify delays in the local blood culture pathway and develop quality improvement measures to improve and re-audit these.
2. Audit of the total TAT from specimen collection to clinical action and analysis of delays in molecular and biomarker testing. Identify delays in the local pathway and develop quality improvement measures to improve and re-audit these.
3. Audit of blood volume collected for culture in adults.
4. Audit of the impact of blood culture results on antimicrobial prescribing
5. Audit of key performance indicators if they focus on value to patient, rather than on technical detail.
6. Audit of appropriateness of the requests for molecular, biomarker tests and impact on antimicrobial prescribing

Recommendation for future research

- Prospective study on the impact of various delays in the blood culture pathway on patient care and outcomes

10 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Appendix A: Critical control points in blood culture investigation

By breaking down the blood culture process, it is possible to identify critical control points where there may be delays or the potential to improve turnaround times (TAT) (55).

The term TAT, in this context, refers to the time taken from blood culture collection to the time of reporting. Laboratory TAT refers to the time from receipt of the sample in the laboratory to reporting of results. The time taken to achieve each of the following stages of the process has an effect on the overall TAT:

- time from collection to receipt within the laboratory (55),(97) — transport time (TT)
- time from receipt to loading on blood culture analyser (55)
- time from loading to registering positive — time to detection (TTD) (134) (135)
- time from flagging positive to identification and susceptibility results (59). For samples loaded on remote analysers in satellite laboratories in hospitals without on site laboratories, this stage will include the time taken to convey the flagged bottle to the main laboratory
- time from identification and susceptibility results to reporting

The time from collection to loading is dependent on many factors:

- the location of the laboratory in relation to the ward (onsite or offsite)
- external transportation arrangements (frequency, out of hours service)
- internal transfer arrangements (frequency, availability of pneumatic tube transport, out of hours service)
- level of laboratory out of hours service provision (out of hours loading frequency)
- equipment available and developments in current technology (availability of continuous monitoring blood culture analyser)

A decrease in the time to positivity (time from collection to flagging positive) can be achieved in a number of ways depending on local facilities and resources (97):

- external and internal transport arrangements to decrease collection to loading time
- shift working patterns or out of hours laboratory cover to decrease collection to loading time
- use of non microbiology (for example blood sciences) staff to load machines out of hours
- use of automated analysers located in a remote location within hospitals without on site laboratories. Prior to installation, careful analysis of specimen processing workflow would need to be undertaken to ensure that delays with processing of blood culture bottles that have flagged positive do not outweigh any benefits from earlier commencement of incubation.

- new developments in current technology which decrease the collection to loading time and time to positivity

Excluding the time from placement on the blood culture machine to detection (TTD), each stage of the process is dependent on multiple external factors including transport infrastructure, prioritisation and speed of processing by staff, out of hours service delivery and timely communication of positive identification and susceptibility results to medical staff. Once the culture is placed on the blood culture machine, there is little that can be done to speed up the process until sufficient growth has occurred for the bottles to flag positive. The time from flagging a positive result to identification and susceptibility results can be further subdivided in 2 stages; the time from flagging positive to removal from the culture machine, and the time from removal to results of Gram stain, identification and sensitivities. Preliminary results may be given verbally prior to final report generation.

Every laboratory will have to determine the cost effectiveness of any necessary investment to achieve clinical benefit in terms of clinical outcome and antimicrobial stewardship. In the event that the laboratory is unable to ensure timely incubation of blood cultures due to transport constraints, robust mitigations should be put into place. These may include comprehensive written antimicrobial guidance and availability of consultations by infection specialists. If satellite incubation units are used, systems should be implemented that minimize delays with transportation of flagged bottles to the main laboratory.

Timeline - Critical Control Points

Decreasing TAT has the potential to improve clinical outcomes because positive blood culture results provide a second opportunity via reports and clinical liaison to optimise antibiotic treatment where initial empirical therapy has been suboptimal (52,54).

Appendix B: Recommended audit standards

The recommendations for loading of blood culture bottles on automated monitoring analyser and processing of positive blood cultures are deemed good standards of practice to achieve. Laboratories that are unable to achieve these standards may be expected to provide a justification for their practice, with a suitable risk assessment.

To optimise the clinical utility of blood culture results, the interval between collection of samples and reporting of results should be kept to a minimum. The recommended turnaround time (TAT) from collection to reporting is between 1 and 5 days (longer if fungal infection is suspected, if extended incubation is required, or if isolates are sent to a reference laboratory for confirmation) (136). By breaking down the blood culture process, it is possible to identify critical control points where there may be delays or the potential to improve TATs (Appendix A). This has the potential to lead to improved patient outcomes, however robust data are limited (55). There is also the potential to enable earlier optimisation of antimicrobial use, although robust data are also lacking (28). The process can be subdivided into pre analytical, analytical and post analytical phases, all of which should be completed within the recommended time frame.

Once implemented, standards should be audited regularly to ensure that they are met and to evaluate current service provision. These standards are designed to emphasise the critical nature of the blood culture specimen for patient management; they do not assume that the pathology service is required to invest in specific equipment but encourage the optimal use of the resources already in place. Laboratories that are unable to meet these standards without significant additional resource should undertake a formal risk assessment, balancing any additional clinical outcomes and improvement in antimicrobial stewardship against the required cost.

Summary table 1: Pre analytical standards

Inoculated bottles should be incubated as soon as possible, ideally within a maximum of 4 hours (53-55,97,136).

Investigative stage:	Standard:
Pre analytical	Ideal time period
Collection to Incubation	Less than or equal to 4hours

Summary table 2: Analytical standards

Results of the following identification and sensitivity tests (if performed) should be completed within the following time frames from flagging positive:

Investigative stage:	Criteria:	Standard:
Analytical		
Flagging positive to microscopy, identification and sensitivities	**Test (if test performed)**	**Ideal time period to result**
	Molecular assays	same day
	Isolate identification (Direct or automated)	Less than or equal to 24hours
	Isolate identification (Conventional methods)	24 to 48hours
	Isolate sensitivities (RAST) method (Direct or automated)	8hours
	Isolate sensitivities (Conventional methods)	24 to 48hours

Note that loading may be on to a remote incubator located in a satellite laboratory, hence transport time to the central laboratory will affect the timeframes involved.

Summary table 3: Post analytical standards

Standards have also been set for the laboratory TAT (the time between receipt in the laboratory and reporting):

Investigative stage:	Criteria:	Standard:
Post analytical		
Negative report (from start of incubation in laboratory to negative reporting)	Report type	Ideal turnaround time
	Preliminary negative report	48 hours * (Dependant on local policy)
	Final negative report	Less than or equal to 5 days of completed incubation (or greater if extended incubation required).
Positive report	Preliminary positive report	Result availability to Infection Specialist or Healthcare

(from receipt in laboratory to positive reporting)	(Release results following local policy: telephone, email or electronic)	professionals caring for the patient: Within 2 hours of identity or sensitivity availability. (following local policy) (see Summary table 2 above)
	Final positive report	Less than or equal to 5 days of completed incubation (or greater if extended incubation required, or if isolates are sent to a reference laboratory for confirmation)

*Refer to neonatal sepsis section of the background for further information regarding negative reporting of neonatal blood culture.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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