UK Health Security Agency

UK Standards for Microbiology Investigations

Sepsis, and other systemic and disseminated infections

This draft document was consulted on between 15 November 2021 to 29 November 2021

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

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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@phe.gov.uk.

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

Amendment number / date	
Issue number discarded	
Insert issue number	
Anticipated next review date*	
Section(s) involved	Amendment

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

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1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

3.1 Scope

This document describes relevant investigation that should be considered with different presentations of sepsis, and other systemic and disseminated infections.

In certain circumstances, particularly in the profoundly immunocompromised host, fungal and viral infections are important causes of systemic infection. This UK SMI includes guidance on the initial investigation.

Validated diagnostic tests on positive blood cultures are available and should be considered for use.

The UK SMI does not address the detection of parasites, viral load testing or *Mycobacterium* species and does not list specific details of commercially available systems.

Please see B 40 – Investigation of specimens for *Mycobacterium* species and V 28 – Investigation of cytomegalovirus infection.

This UK SMI should be used in conjunction with other UK SMIs.

4 Background

The detection and culture of micro-organisms from blood, serum, CSF, urine, BAL and skin samples are essential for microbiological diagnosis of bacteraemia, fungaemia (particularly candidaemia), infective endocarditis and conditions associated with a clinical presentation of pyrexia of unknown origin (1,2). Blood culture is also important for the diagnosis of prosthetic device infections such as vascular grafts and intravascular line associated sepsis. Blood cultures may also detect infections associated with other conditions such as pneumonia, septic arthritis and osteomyelitis.

Antibiotic resistance (3 to 5) amongst pathogens (particularly Gram negative bacteria) is an important cause of ineffective empirical treatment of bloodstream infections. Early identification and antibiotic susceptibility results for blood culture isolates provide valuable diagnostic information on which appropriate antimicrobial therapy can be based, so helping to reduce morbidity and mortality, improve patient care, support antimicrobial stewardship and reduce healthcare costs (7 to 10). Decreasing turnaround times (TAT) at each stage of the process from transportation of samples to reporting of results is therefore recommended (1).

Increasing numbers of commercial systems are becoming available that can detect specific bacterial and fungal pathogens and their more important resistance mechanisms more rapidly than traditional technologies.

In some clinical settings, namely critically ill and significantly immunocompromised patients, targeted investigation for certain viral and fungal causes are indicated.

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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 (11,12). 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 (7). 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 (13,14).

4.1.1 Transient

The transient presence of bacteria or fungi 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 characteristically 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.

4.1.3 Continuous

Continuous bacteraemia 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 (15,16) and pseudofungaemia (17) with environmental organisms have been described involving contaminated equipment on wards and laboratories.

4.2 Sepsis

4.2.1 Sepsis and septic shock

Sepsis is defined as life threatening organ dysfunction caused by dysregulated host response to infection (18 to 21). 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 (22).

4.2.2 Neonatal sepsis

Neonatal sepsis (23 to 25) may occur within the first 4 weeks of life. The incidence of neonatal sepsis increases with low birth weight or prematurity and can be divided into early onset and late onset.

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

4.3 Organisms implicated

4.3.1 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. 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 bacteraemic 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*

Organisms associated with zoonoses include *Pasteurella* species, *Capnocytophaga* species and *Brucella* species.

4.3.2 Healthcare associated infection (HCAI)

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

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 (26):

- *E. coli* and other Enterobacterales
- *S. aureus*
- coagulase negative staphylococci
- *Pseudomonas aeruginosa*
- Enterococci
- anaerobes
- *Candida* species and other yeasts

Many other organisms have been implicated in both hospital and community acquired bacteraemia (27 to 35).

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4.3.3 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 fetus (36,37). 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 (36).

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.

4.3.4 Neonates

Organisms associated with neonatal sepsis include (23,24):

- 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* species and other yeasts

Group B streptococci and *E. coli* are major causes of early onset neonatal sepsis (38).

Neonatal sepsis caused by anaerobic bacteria has been reported; the majority of cases being due to *Bacteroides* species, *Clostridium* species or *Peptostreptococcus* species(39).

4.3.5 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 (40).

Transient bacteraemia can occur in children with few or none of the symptoms normally associated with bloodstream infection (41). *S. pneumoniae* predominates, but occult infection with *H. influenzae*, *Salmonella* species and *N. meningitidis* has also been described.

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4.3.6 Infective endocarditis (IE)

IE is defined as an infection of the heart valves or other areas of the endocardium (42). Infection can occur with both native valves and prosthetic valves. The Duke criteria are used for diagnosis (43). Historically, the disease was classified as either 'acute' or 'subacute' (44); however, it is more usual to describe the disease in relation to the infecting organism or the underlying anatomy. Endocarditis caused by *C. burnetii*, can be detected in blood.

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 bacteraemias.

Organisms most commonly isolated include (45):

- oral streptococci
- Staphylococci (especially *S. aureus (44)*)
- Enterococci
- *Streptococcus bovis (S. bovis* biotype 1 may also be referred to as *S. gallolyticus subsp. gallolyticus)* (46)

Fungal infectious endocarditis is rare, except in people who inject drugs and patients with severe underlying illnesses, and requires immediate treatment or surgery (47,48). 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 ID 12 – Identification of Haemophilus species and the HACEK group of organisms) (43,49). 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 systems (49 to 51). *Bartonella* species have been implicated as causes of endocarditis with severity of infection correlating with immune status.

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 (45). IE involving the prosthetic aortic valve is more common than that involving prosthetic mitral, tricuspid or pulmonary valves (52).

'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 in native valve endocarditis (44). 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 (45):

- coagulase negative staphylococci
- *S. aureus*
- Gram negative rods

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- *Candida* species
- Streptococci and enterococci
- *Corynebacterium* species

4.3.7 Anaerobic bacteraemia

Anaerobes are an important cause of bacteraemia and are to be tested for routinely (39,58 to 60). Organisms most commonly associated with anaerobic bacteraemia include (39):

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

4.3.8 Immunocompromised patients

In patients who are immunocompromised, there is a high incidence of infection caused by organisms that are typically non virulent and that form part of the normal host and environmental flora (53). Immunocompromised patients range from the critically ill to those with aplastic neutropenia, where the risk of infection is greatest and in whom Gram negative bacteria cause severe sepsis associated with a high mortality rate (53).

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 (35,54).

The spectrum of organisms detected reflects lengthening periods of neutropenia and duration of hospital stay, and an 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 (35). In addition to the organisms associated with bloodstream infection in the immunocompetent, isolates include (54):

- Non fermentative Gram negative rods, particularly *P. aeruginosa (*55)
- *Listeria monocytogenes*
- *Corynebacterium* species
- *Candida* species 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 (54,56,57).

4.3.9 Fungaemia

Systemic fungal infections are typically caused by opportunistic fungal pathogens in immunocompromised hosts. However, invasive disease can occur in immunocompetent

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individuals if the exposure is high or with primary (dimorphic) fungal pathogens (causes of endemic mycoses including Coccidioides and Talaromyces spp.). Systemic fungal infections originate either in the lungs (Aspergillus, Cryptococcus, Mucorales, as a result of inhalation) or from endogenous flora (Candida spp. 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) whereas invasive Aspergillus sp or Pneumocystis jirovecii infections cannot be detected by blood culture. Therefore, 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 sensitivity 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.

Candidaemia is a diagnostic challenge as the concentration of Candida in the blood during candidaemia is low with up to 65% of positive blood cultures having less than 1 colony forming unit per millilitre. In addition, Candida rapidly forms biofilms and is released into the bloodstream only intermittently and transiently. The mean sensitivity of blood culture is less than 40% (autopsy-proven cases), and 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%. The low concentration and intermittent presence of Candida is also a major challenge for the polymerase chain reaction (PCR). A number of Candida PCR platforms are available commercially for testing whole blood, serum or plasma.

Detection of fungal antigens such as β-1,3-D-glucan (BDG) in serum or sterile body fluids is a sensitive but non specific indicator of fungaemia. BDG is present on most fungal pathogens with the exception of Mucor, Rhizopus, Blastomyces and some other rare species and it is thus commonly called a pan fungal biomarker. Among high risk patients, an initial measurement of BDG greater than 80 pg/mL is strongly associated with the occurrence of invasive candidosis, pneumocystosis or aspergillosis although false positive results occur. On the other hand, 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 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 sp.

4.3.10 Viral sepsis

Almost any virus has the potential to cause viral sepsis in a susceptible population. For example 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. Many viruses have the ability to cause sepsis in the immunosuppressed

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individual, for example, HSCT and SOT recipients are at high risk of infection (primary infection or reactivation) with 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 RSV, parainfluenza and adenovirus are cause for concern in these patient groups.

Molecular detection of viruses in blood, respiratory specimens, CSF, skin swabs or faeces 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 assays may be employed as relevant to clinical indications.

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 contribute 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.

4.4 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 (61); however, they may be useful in the investigation of sudden unexpected death in infants and children (SUDI) (6,62 to 64).

4.5 Automated blood culture systems

The ideal blood culture system produces the maximum yield of pathogen in as short a time as possible in order to have the greatest influence on patient management (65). The introduction of fully automated, continuous monitoring blood culture systems has led to earlier detection of pathogens (11).

Blood cultures should 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.

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 (66,67). As a result, many laboratories now store and transport delayed samples at room temperature overnight (68 to 70).

A balance between obtaining false negative blood culture and incurring significant delays in the Gram stain result must be carefully considered as it is estimated that 2 to 5% of positive samples may be missed if bottles are pre incubated; 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 (68,71 to 73).

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 (68).

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4.6 Molecular technologies

Laboratories may consider the use of molecular tests at various stages of investigation depending on what systems are available. Systems may be available that operate directly on inoculated culture bottles, either before incubation or following a positive flag. Systems that detect the most common antibiotic resistance genes are available (2 to 4). Local protocols should be developed to determine when molecular tests should be undertaken.

4.7 Factors affecting isolation of causative organisms

A number of clinical factors may affect the isolation of the infecting organism, regardless of the system employed (11,35).

Method of collection

Studies 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 (74,75). 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 (76). 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 (16,77 to 80).

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% (81 to 83). 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) (80,84). 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 (82,84,85).

Number and timing of samples

For the majority of patients, 2 blood culture sets are recommended. A second or third set taken from a different site not only increases yield but also allows recognition of contamination (86). In most conditions other than endocarditis, bacteraemia is intermittent, given it is related to the fevers and rigors which occur 30 to 60 minutes after the entry of organisms into the bloodstream. Samples should be taken as soon as possible after a spike of fever. However, 1 study has shown no significant difference in isolation rates for blood drawn either at intervals or taken simultaneously with fever spikes (87). Timing of sample collection may be less important in cases of continuous bacteraemia.

Volume of blood

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,

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with approximately a 3% increase in yield per mL of blood cultured. False negatives may occur if inadequate blood culture volumes are submitted (90).

The number of organisms present in adult bacteraemia is frequently low, often less than 1×10^3 colony forming units per litre (cfu/L) (91). For adult patients it is recommended that 20 to 30mL of blood be cultured per set (59,92). Most modern commercial systems 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 volumes is often based on weight rather than age and relates to total patient blood volume (90). 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. It has been suggested that the volume of blood drawn should be no more than 1% of the patient's total blood volume (93,94).

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, not 1%, should be cultured (95).

Automated blood culture systems may offer the facility to monitor bottle weight or to estimate volume of blood.

Previous antimicrobial therapy

Ideally, blood samples should be taken prior to antimicrobial treatment. Any recent antimicrobial therapy can have a significant effect on blood culture results by decreasing the sensitivity of the test, which may result in false negatives. If patients have received previous antimicrobial treatment, bacteraemia or candidaemia should be considered even if blood culture results are negative.

Media containing antibiotic inactivating resins and other adsorptive materials including charcoal have been developed to overcome the effect of antimicrobials (88,89). Some media, however, rely on optimal blood broth dilution for antimicrobial neutralisation (89).

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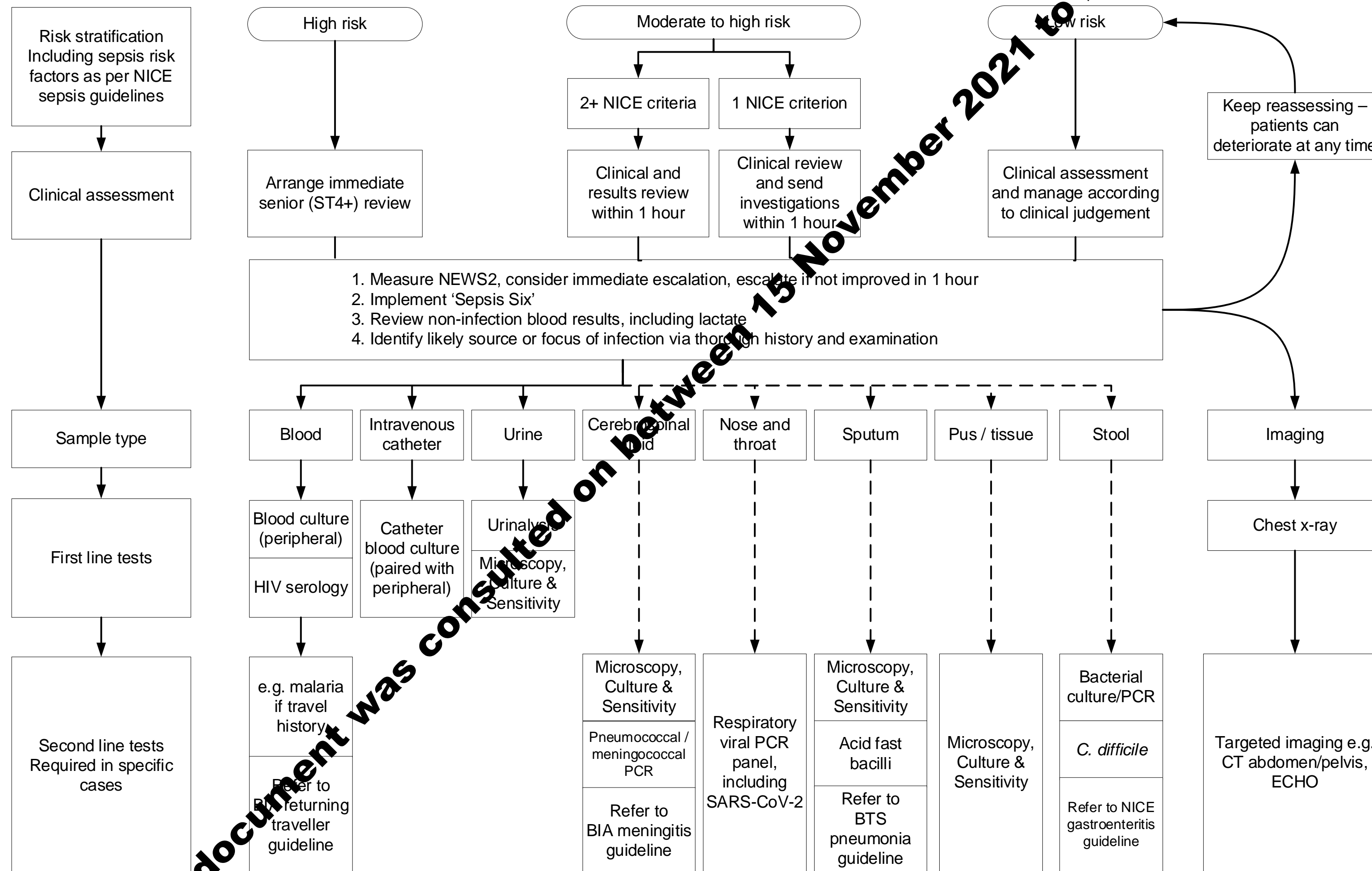
4.8 Algorithm 1: investigation of sepsis in adult hospital patients

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. An accessible text description of this flowchart is provided with this document



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Footnotes on 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.

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 apart from blood culture are generally not needed.

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.

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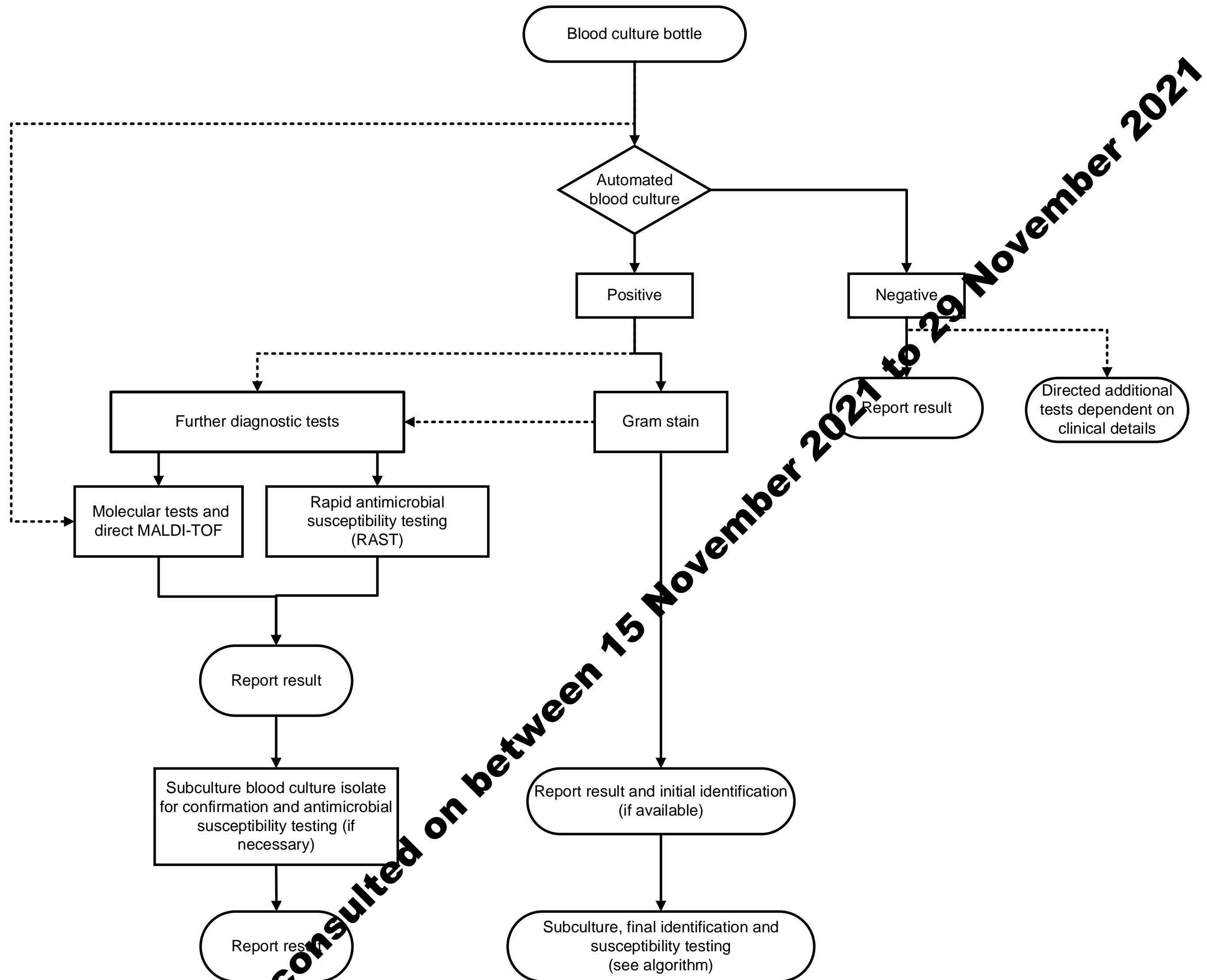
4.9 Algorithm 2: Investigation of blood cultures bottles

An accessible text description of this flowchart is provided with this document



Footnotes on algorithm 2

- Laboratories may consider the use of molecular tests at several stages depending on what system is available. Systems 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, 18S sequencing) 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, *Bartonella* serology) (170). Serologic testing may also be helpful if exposures are suggestive of *Brucella* endocarditis

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- Serological testing of (1,3) —Beta-D—Glucan may be used as a rule out test for candidaemia; false positives are common hence results should be interpreted as part of the wider clinical context
- Reasons for negative blood cultures include concomitant or antecedent antibacterial 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

5 Pre laboratory processes (pre analytical phase)

5.1 Specimen type

The types of specimens include:

- Blood for
  - Blood culture. Other specimens may be processed in blood culture bottles where appropriate (see B 26 – Investigation of fluids from normally sterile sites and B 38 – Investigation of bone marrow).
  - NAATs — viruses, bacteria (especially TB) and fungi
- Serum for
  - Fungal biomarker tests. Follow manufacturers' instructions. 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
  - Virus serology
- CSF for
  - Bacterial and fungal culture (see B 26 – Investigation of fluids from normally sterile sites)
  - NAATs — viruses, bacteria (especially TB), fungi, 16S and 18S sequencing
  - Fungal biomarker and cryptococcal antigen tests
- Urine for
  - Urinalysis and culture
  - Bacterial antigen tests (pneumococcus, legionella)
- BAL for
  - Bacterial and fungal culture
  - NAATs — viruses, bacteria (especially TB), fungi, 16S and 18S sequencing
  - Fungal biomarker tests
- Blister swabs, skin biopsies and other tissue for
  - NAATs for viruses and 16S and 18S sequencing (as appropriate)
  - Bacterial and fungal culture

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5.2 Specimen collection, transport and storage

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers (according to manufacturers' instruction if using a continuous monitoring blood culture system) and transport in sealed plastic bags. Appropriate blood culture bottles must be used for specific machines when using continuous monitoring blood culture systems 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 (96 to 103).

Collection of blood from the patient should be carried out following Department of Health guidance (81).

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

Compliance with postal, transport and storage regulations is essential.

For safety considerations refer to section 2: scientific information in this document.

Collect specimens before antimicrobial therapy where possible (103).

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, except in endocarditis where timing is less important (87).

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.

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.

5.3 Adequate quantity and appropriate number of specimens

A blood culture set is defined as 1 aerobic and 1 anaerobic bottle. Some proprietary media collection systems may be presented as a single bottle; manufacturer's guidance on volume should be followed (103).

For infants and neonates, a single aerobic bottle is usually requested.

Quantity

Adults

Preferably, a volume of 20 to 30mL for each blood culture set should be taken.

Children and neonates

No more than 1% of the total blood volume.

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Note: Do not exceed the manufacturer's recommended maximum volume for each bottle. Different manufacturers market different bottle formats.

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.

Number

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

Take 2 consecutive sets from 2 separate venepuncture sites (81) during any 24hour period for each septic episode (86). For neonates, take a single aerobic bottle or special low volume bottle.

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 (81).

Take 2 sets during the first hour in cases of sepsis prior to commencing antibiotic treatment, provided this does not significantly delay antibiotic administration (18).

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

5.4 Optimal transport and storage conditions

Specimens should be transported and processed as soon as possible (96,97,103).

Inoculated bottles should be loaded on to automated blood culture systems (or incubated in the case of non automated systems) without delay, and ideally within a maximum of 4 hours from time of collection (65,68,72,73,104).

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

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens' (105).

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 (106).

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 (69,70).

5.5 Specific safety considerations

Containment Level 2.

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*) is suspected, or when subculturing blood culture bottles from suspected cases of typhoid or paratyphoid fever. 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) must be conducted in a microbiological safety cabinet (MSC) (181).

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Ideally all blood cultures should be sub cultured 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 grow as yeast forms in blood culture bottles and sub cultures at 37°C, but as the highly infective mould form when sub cultured to agar incubated at 28 to 30°C. Care should be taken with yeast isolates if there is a relevant travel history, especially in the immunocompromised.

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.

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

6 Laboratory processes (analytical phase)

Blood Culture bottles

6.1 Specimen processing or procedure

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 systems.

6.1.2 Sample preparation

Pre treatment

NA

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 systems

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

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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 (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 (96 to 101,107 to 117).

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 (118).

Positive bottles from manual systems

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

Negative bottles from continuous monitoring systems

Blind subculture bottles from patients if clinically indicated.

Negative bottles from manual systems

Perform blind subculture for any aerobic bottle that appears negative after 24 to 48 hours (119).

Supplementary

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

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 Technical Information and Limitations for further information.

Manual systems or automated system failure

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

6.2 Microscopy

Positive bottles for all systems

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 systems 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.

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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 (120) (TP 39 – Staining procedures).

If organisms are not seen on microscopy:

1. Investigate the growth curve (automated systems). 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 system, according to manufacturer's instructions, for further incubation and testing.
3. Consider *Mycobacterium* species. B 40 – Investigation of specimens for *Mycobacterium* species.

On automated systems false positive signals may be caused by excess blood volume or a high white cell count. Manufacturers recommendations should be followed.

6.3 Culture and investigation

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

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

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6.3.1 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

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Systemic fungal infection#	Blood	Sabouraud agar	28 to 30	air	5d	2d and at 5d	Yeasts 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. # 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 (121 to124). ‡other organisms may need to be considered.							

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

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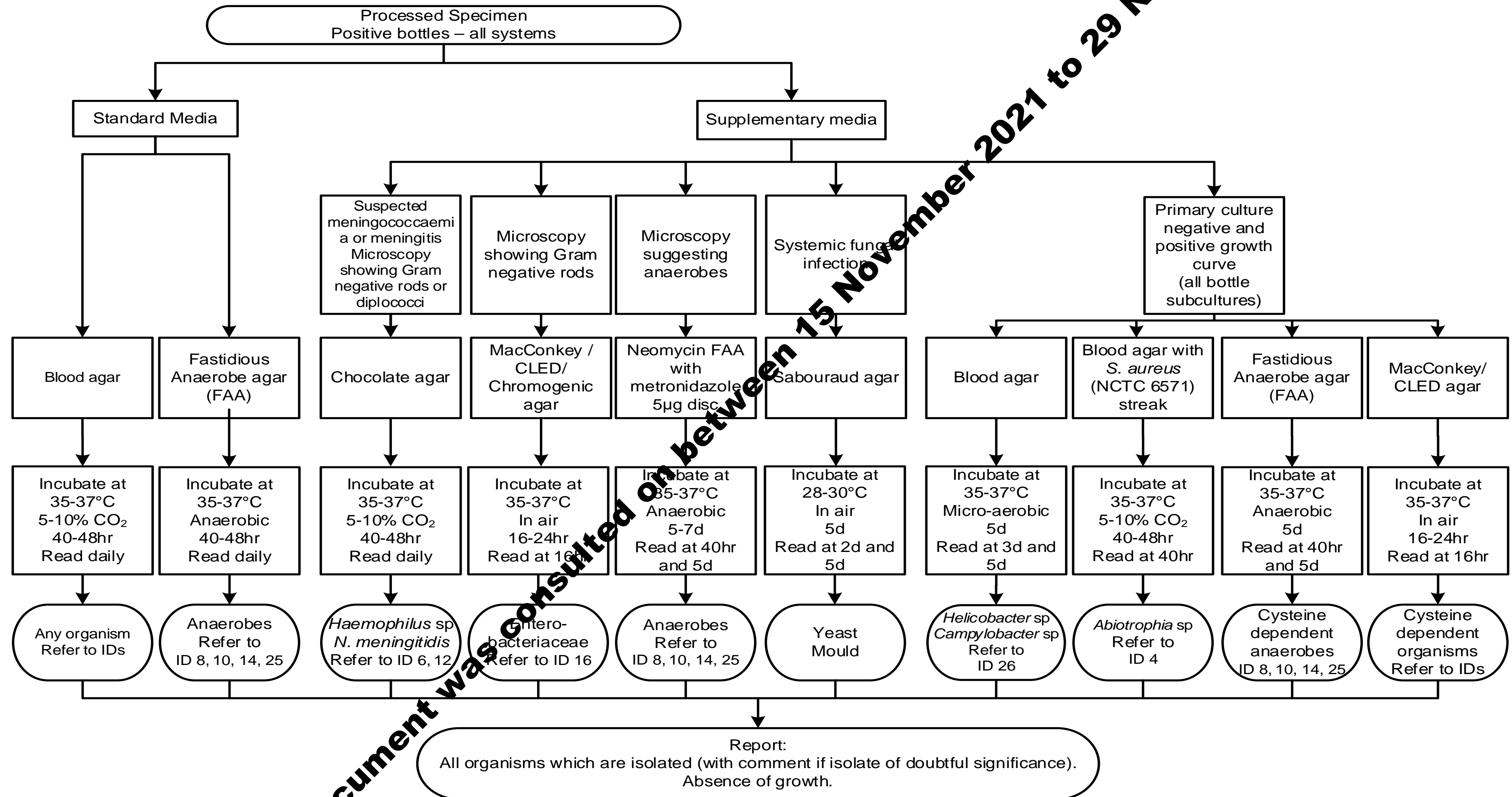
6.3.2 Algorithm 3: Culture media, conditions and organisms for investigation of blood cultures (for organisms other than Mycobacterium species)

An accessible text description of this flowchart is provided with this document



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Serum for fungal biomarker tests

Commercial kits for detecting Candida specific antigens are available. Measurement of galactomannan in serum or CSF is the most commonly used test to diagnose invasive aspergillosis. Follow manufacturer's instructions.

Specimens for fungal NAATs testing

A number of Candida PCR platforms are available commercially for testing whole blood, serum or plasma. Follow manufacturer's instructions.

6.4 Identification

Refer to individual UK SMIs for organism identification.

Minimum level in the laboratory

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.

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).

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 (5,125 to 128).

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) RAST method (129) 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 in line with recommendations from EUCAST. If it is not possible to test in house then this should be referred.

6.6 Rapid methods

6.6.1 Nucleic acid amplification tests

Use of polymerase chain reaction (PCR) tests and other nucleic acid amplification tests (NAATs) for identification of bacteria from positive blood samples (131 to 133) 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 (134). Several assays are available including pathogen specific assays, broad range assays and multiplex assays (1,5,7,53,130,38).

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6.6.2 MALDI-TOF mass spectroscopy

The use of matrix assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectroscopy (134) in the identification of organisms directly from positive blood culture and blood culture isolates has been evaluated (128,134 to 139). It is possible for an organism to be identified from an isolate within a short time frame and it is commonly used in laboratories to provide a robust identification system. Direct identification of Gram positive bacteria (particularly staphylococci) may be less reliable than Gram negative bacteria and that media composition (for example inclusion of charcoal) may affect identification (134 to 138).

Local protocols should be developed to determine when rapid testing should be undertaken.

Refer to UK SMI TP 40: MALDI TOF MS test procedure for further information on direct identification.

6.7 Specific technical limitations

6.7.1 Terminal subculture

Terminal subculture is not normally required in automated systems. If manual or semi automated systems 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.

6.7.2 Intravenous catheter related bacteraemia

Differential time to positivity, and differential quantitative culture as a means of diagnosing catheter related bacteria is not recommended by this UK SMI due to issues with reliability (80,140 to 145), and its lack of applicability to cases of polymicrobial infection(140).

6.7.3 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 (146 to 148). 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* (118). 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.

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

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

Reasons for false positivity are often multifactorial. On automated systems 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 systems, 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, Giemsa or Sandiford may be required to demonstrate the presence and morphology of the organisms involved (120). 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.

6.7.4 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 (88,89,149 to 151). 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 systems (152,153). 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* (11,154 to 156).

6.7.5 Incubation time and temperature

A temperature of 35 to 37°C for 5 to 7 days is recommended for routine blood cultures (11). Five days is usually sufficient incubation time for the recovery of most organisms if automated systems are used (157,158). If a shorter incubation duration is used, such as 4 days, laboratories must validate their processes (162). 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 (159 to 161). 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 (163). 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 (7,11,49,50,164).

7 Post laboratory processes (post analytical phase)

Locally agreed policies for the release of results should be written based on local LIMS and user requirements.

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7.1 Microscopy reporting

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 Culture reporting

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 (8).

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) (25,165). 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 or according to local protocols.

7.3 Molecular reporting

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.

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7.4 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

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

England and 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 PHE under The Health Protection (Notification) Regulations 2010.

Other arrangements exist in Scotland (166),167, Wales (168) and Northern Ireland (169).

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Appendix 1: 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) (2).

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 (2,6) — transport time (TT)
- time from receipt to loading on blood culture system (2)
- time from loading to registering positive (97) — time to detection (TTD)
- time from flagging positive to identification and susceptibility results (4,24). 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 system, pre incubation incubator)

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 (65):

- 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

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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 a 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 improved 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 (25,35). Unfortunately, robust evidence is lacking at the time of writing (2).

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Appendix 2: Recommended audit standards

The recommendations for loading of blood culture bottles on automated monitoring equipment 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) (104). 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 1). This has the potential to lead to improved patient outcomes, however robust data are limited (68). There is also the potential to enable earlier optimisation of antimicrobial use, although robust data are also lacking (2,26,35). 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 (106). 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 (65,68,72,73,104).

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 (38,131,171 to 173):

Investigative stage:	**Criteria:**	**Standard:**
Analytical		
Flagging positive to microscopy,	**Test** (if test performed)	**Ideal time period to result**
	Molecular assays	same day

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identification and sensitivities	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) (11,25,123,157 to 161):

Investigative stage:	Criteria:	Standard:
Post analytical		
	Report type	**Ideal turnaround time**
Negative report (from receipt in laboratory to negative reporting)	Preliminary negative report	48 hours * (dependant on local policy)
	Final negative report	Less than or equal to 5 days (or greater if extended incubation required)
Positive report (from receipt in laboratory to positive reporting)	Preliminary positive report (Release results following local policy: telephone, email or electronic)	Result availability to consultant microbiologist: Within 2hours of identity or sensitivity availability. (following local policy) (see Summary table 2 above)
	Final positive report	Less than or equal to 5 days (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 (25,165).

This draft document was consulted on between 15 November 2021 to 29 November 2021

References

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

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