UK Standards for Microbiology Investigations

Investigation of Blood Cultures (for Organisms other than *Mycobacterium* species)

Issued by the Standards Unit, Microbiology Services, PHE

Acknowledgments

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UK Standards for Microbiology Investigations[#]: Status

Users of SMIs

Three groups of users have been identified for whom SMIs are especially relevant:

- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
- SMIs also provide commissioners of healthcare services with the standards of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe essential laboratory methodologies which underpin quality, for example assay validation, quality assurance and understanding uncertainty of measurement.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health interventions, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

Involvement of Professional Organisations

The development of SMIs is undertaken within PHE in partnership with the NHS, Public Health Wales and with professional organisations.

The list of participating organisations may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of an organisation's logo in an SMI implies support for the objectives and process of preparing SMIs. Representatives of professional organisations are members of the steering committee and working groups which develop SMIs although the views of participants are not necessarily those of the entire organisation they represent.

SMIs are developed, reviewed and updated through a wide consultation process. The resulting documents reflect the majority view of contributors. SMIs are freely available to view at http://www.hpa.org.uk/SMI as controlled documents in Adobe PDF format.

[#] UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

The process for the development of SMIs is certified to ISO 9001:2008.

NHS Evidence has accredited the process used by PHE to produce SMIs. Accreditation is valid for three years from July 2011. The accreditation is applicable to all guidance produced since October 2009 using the processes described in PHE's Standard Operating Procedure SW3026 (2009) version 6.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are well referenced and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. SMIs should be used in conjunction with other SMIs.

UK microbiology laboratories that do not use SMIs should be able to demonstrate at least equivalence in their testing methodologies.

The performance of SMIs depends on well trained staff and the quality of reagents and equipment used. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

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Microbial taxonomy is up to date at the time of full review.

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Contents

Amendment Table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	10/dd.mm.yy <tab+enter>
Issue no. discarded.	7
Insert Issue no.	xxx
Section(s) involved.	**Amendment.**

Amendment No/Date.	9/27.03.13
Issue no. discarded.	6.1
Insert Issue no.	7
Section(s) involved.	**Amendment.**
Scope.	Standards for turnaround times of blood culture developed and added to Scope. Cross reference to blood borne viruses in dialysis patient added.
Introduction.	Reorganisation of text. Sepsis section updated to include SIRS and neonatal sepsis. Addition of Healthcare Associated Infection. Removal of differential quantitative culture for confirmation of catheter related bacteraemia. Unusual organisms section streamlined, links updated. Section on antibiotic resistance added. Section on blood culture systems updated to include limitations and suggestions for service improvement. Factors affecting isolation of causative organisms updated: recommended blood volumes for children removed, incubation time and temperature updated. Rapid identification and sensitivity section: Recommendation for direct sensitivity testing - 'it is recommended that direct

	sensitivity tests are performed on positive blood culture bottles where appropriate'. Method information expanded to include automated and molecular methods and MALDI-TOF. Contamination target rate <3%.
Technical Information/Limitations.	Updated to include information regarding pre-incubation of blood culture bottles.
Specimen Collection, Transport and Storage.	Updated to reflect standards in scope. Recommendation added for laboratory management to establish and manage transportation of samples to the laboratory. Correct specimen type and method of collection updated to reflect Department of Health guidelines.
Specimen processing.	Updated to reflect standards in scope. 2.5.2 Addition of negative bottles from continuous monitoring systems. 2.5.3 Media for subculture, condition and organism table made consistent with flowchart. 2.8 Addition of links to reference laboratories.
Reporting.	Updated to reflect standards in scope.
Appendix 1.	Critical control points – added.
Appendix 2.	Therapeutic window – added.

Scope of Document

Type of Specimen

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).

Scope

This method describes the processing and microbiological investigation of blood cultures and aims to set standards for each stage of the investigative process.

It does not address the detection of parasites, viruses (for the detection of blood borne viruses in dialysis patients (see V 10 - Blood Borne Virus Testing in Dialysis Patients), or *Mycobacterium* species (see B 40 – Investigation of specimens for *Mycobacterium* species) and does not list specific details of commercially available systems.

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

Standards

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 one and five days (longer if fungal infection is suspected, if extended incubation is required, or if isolates are sent to a reference laboratory for confirmation)[1]. 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[2] (Appendix 1) leading to improved patient outcomes (Appendix 2). The process can be subdivided into pre-analytical, analytical and post-analytical processes, 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[3]. These standards are designed to emphasise the critical nature of the blood culture specimen to patient management; they do not assume that the pathology service needs to invest in specific equipment, but encourage the optimal use of the resources already in place.

Summary Table 1: Pre-Analytical Standards[1,2,4-6]

Inoculated bottles should be incubated as soon as possible, and within a maximum of four hours.

Investigative Stage:	Standard:
Pre-Analytical	Time Period
Collection to Incubation	≤4hr

The four hour turnaround time from collection to incubation for blood culture samples reflects their clinical significance.

Summary Table 2: Analytical Standards[7-13]

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)	**Time Period to Result**
	Gram Stain	≤2hr
	Rapid Antigen Testing	≤2hr
	Molecular Assays	same day
	Isolate Identification (Direct/Automated)	≤24hr
	Isolate Identification (Conventional Methods)	24-48hr
	Isolate Sensitivities (Direct/Automated)	≤24hr
	Isolate Sensitivities (Conventional Methods)	24-48hr

Summary Table 3: Post-Analytical Standards[14-21]

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

Investigative Stage:	Criteria:	Standard:
Post-Analytical		
Negative Report (from receipt in laboratory to negative reporting)	Report Type	Turnaround time
	Preliminary Negative Report	48hr * (dependant on local policy)
	Final Negative Report	≤5 days (or greater if extended incubation required)
Positive Report (from receipt in laboratory to positive reporting)	Preliminary Positive Report (Telephone/Fax/Email)	Immediately, within 2hr of identity/sensitivity availability. (see Summary Table 2 above)
	Final Positive Report	≤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 introduction for further information regarding negative reporting of neonatal blood culture[20].

Introduction

Blood culture is considered to be the “gold standard” investigation for the detection of micro-organisms in blood[22]. The culture of micro-organisms from blood is essential for microbiological diagnosis of bacteraemia, fungaemia, infective endocarditis, and many infective conditions associated with a clinical presentation of pyrexia of unknown origin (PUO)[22,23]. It is also an important component of the diagnosis of prosthetic material infections (eg joints and vascular grafts) and intravascular line-associated sepsis. Blood cultures may also detect bloodstream infection in association with other infectious diseases such as pneumonia, septic arthritis and osteomyelitis.

Antibiotic resistance amongst pathogens (particularly Gram negatives) is the most frequent cause of ineffective empirical treatment in bloodstream infection. Early identification and antibiotic susceptibility of 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 and reduce healthcare costs[24,25]. Decreasing turnaround times (TAT) at each stage of the process from transportation of samples to reporting of results is therefore recommended[22].

Bloodstream Infection

The bloodstream contains many antimicrobial components including lysozyme, leucocytes, immunoglobulin and complement. Organisms may enter the bloodstream from a focus of infection within the body, a surface site colonised with normal flora through broken skin or mucous membrane, the gastrointestinal tract or by the direct introduction of contaminated material to the vascular system[24]. These bacteria are normally removed from the bloodstream within a few minutes; only when the host defences are overwhelmed or evaded does systemic infection become apparent. Mortality is related to the type of infecting organism and the nature of any underlying disease[26,27]. Bloodstream infection is caused by bacteria (bacteraemia) or fungi (fungaemia) in the blood and may be transient, intermittent or continuous[14].

Transient

Transient infection is the presence of bacteria or fungi in the bloodstream for periods of several minutes only. It may follow manipulation of, or surgical procedures in, infected tissue or instrumentation of colonised mucosal surfaces. Common examples include dental extraction and urinary catheterisation. It may also result from chewing especially if dental hygiene is poor. Defecation may also be associated with small numbers of bacteria entering the bloodstream. Pressure on boils or minor skin conditions (eg squeezing spots) can lead to transient bacteraemia. Intravenous drug use may also be a source through contaminated needles or drugs. Transient bacteraemia also occurs in association with localised infections such as pneumococcal pneumonia and pyelonephritis.

Intermittent

Intermittent infection is really "recurrent transient" infection and is characteristically associated with undrained, intra-abdominal abscesses. It occurs early in the course of a variety of systemic and localised infections, eg pneumococcal bacteraemia in pneumococcal pneumonia. Cultures taken during fevers and after the onset of rigors may miss intermittent bacteraemia as bacteria tend to be cleared by host defence mechanisms prior to sampling.

Continuous

Continuous bacteraemia suggests a severe infection that has overwhelmed host defences. It is also characteristic of intravascular infection eg infective endocarditis and suppurative

thrombophlebitis. Occasionally, continuous bacteraemias occur in association with non-vascular sources, especially in patients who are immunosuppressed.

Pseudobacteraemia

Pseudobacteraemia occurs when blood culture isolates originate from outside the patient's bloodstream. Blood culture contamination may occur at any stage between taking a blood sample and processing in the laboratory, and can originate from a variety of sources. Outbreaks of pseudobacteraemia have been described involving contamination of fluids and equipment on wards and laboratories with environmental organisms and incorrect sampling of blood[28,29].

Sepsis[30]

The term Systemic Inflammatory Response Syndrome (SIRS) describes the early response of the body to injury and may be infective or non-infective in origin[31]. SIRS is present when two or more of the following clinical features are present[31]:

- Body temperature <36°C or >38°C
- Heart rate >90 beats per minute
- Hyperventilation >20 breaths per minute
- White blood cell count >12,000 cells per µL or <4000 cells per µL

Sepsis, previously referred to as septicaemia, describes a patient with SIRS in whom the cause is infection. It is defined as infection plus systemic response to, or manifestations of, infection[30,31]. Around 20% of sepsis cases are associated with bacteraemia, the rest are secondary to infection at other sites in the body[31]. The incidence of sepsis continues to rise with a reported associated mortality rate of 35-65%[32]. Early appropriate empirical antibiotic treatment is associated with decreased mortality rates and improved clinical outcomes[25,32]. In severe sepsis each hour of delay in antibiotic treatment results in an increase in mortality[33,34].

In the immunocompromised host sepsis is defined as SIRS with one or more of the clinical features present, combined with an infective aetiology.

Severe Sepsis

Severe sepsis is defined as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion[30].

Septic Shock

Septic shock is defined as sepsis-induced hypotension persisting despite adequate fluid resuscitation[30].The clinical symptoms are usually due to toxic bacterial products, the host response to these, or both. Shock is more commonly seen with Gram negative septicaemia, but shock may also be associated with Gram positive organisms, particularly with fulminant pneumococcal, Lancefield Group A streptococcal and staphylococcal bacteraemia[35].

Antimicrobial agents are of little help in combating the acute effects of shock; intravenous antibiotic therapy within the first hour of recognition of septic shock and severe sepsis is recommended[30]. Other supportive measures, such as fluid therapy, mechanical ventilation and the maintenance of blood pressure, are essential.

Neonatal Sepsis

Neonatal sepsis is defined as clinically diagnosed SIRS caused by infection occurring within the first four weeks of life. The incidence of neonatal sepsis increases with low birth weight or prematurity and can be divided into two types:

Early onset neonatal sepsis[20,36]

Early onset neonatal sepsis occurs in the first 72 hours of life and is usually caused by infection ascending from the maternal genital tract or, less commonly, via the placenta.

Late onset neonatal sepsis[36]

Late onset neonatal sepsis occurs after the first 72 hours of life and the organisms may be acquired from the external environment (eg hospital or home). Infection is often transmitted via the hands of care providers; organisms initially colonise superficial sites and the upper respiratory tract and progress to cause widespread sepsis, pneumonia or meningitis.

Organisms isolated from superficial sites, 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. Isolation of organisms from blood remains the gold standard method of diagnosing systemic bacterial infection in neonates. Organisms associated with neonatal sepsis include[36,37]:

- β-haemolytic streptococci, in particular Lancefield group B streptococci
- *Enterobacteriaceae*
- *S. aureus*
- Coagulase negative staphylococci
- *Listeria monocytogenes*
- *Enterococcus* species
- Pseudomonads
- Yeasts

Neonatal sepsis caused by anaerobic bacteria has been reported the majority of cases being due to *Bacteroides* species, *Clostridium* species or *Peptostreptococcus* species[38].

Surveillance screening is performed routinely in many neonatal units and may be used to monitor trends in resistant flora and define antibiotic policies[39,40].

Negative blood culture results at 36hr after collection may be used as a basis for discontinuation of antibiotic treatment following NICE guidance on antibiotic use in early onset neonatal infection. It has been suggested that 36hr is sufficient incubation to rule out sepsis in asymptomatic neonates, however blood cultures collected from neonates < 72hr old may require longer incubation[20,41,42].

Bloodstream Infections in Patients who are Immunocompetent

Community acquired

Community acquired bacteraemia and fungaemia often arises 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 (as is sometimes the case in *Staphylococcus aureus* osteomyelitis). Other generalised bacteraemic illnesses include enteric fever (eg typhoid) and brucellosis.

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

- *Escherichia coli*

- *Streptococcus pneumoniae*
- *S. aureus*
- Other *Enterobacteriaceae*
- *Neisseria meningitidis*
- β-haemolytic streptococci

Hospital acquired

The increasing number of invasive procedures such as catheterisation, immunosuppressive therapy, antibiotic therapy, and life support measures has resulted in an overall increase in hospital acquired bacteraemia, candidaemia and other fungaemia. These procedures may introduce organisms to the bloodstream or may weaken host defences. Organisms most frequently isolated from adults with hospital acquired bloodstream infection will depend on the patient group and may change with the duration of stay in hospital. Organisms include[43,44]:

- Coagulase negative staphylococci
- *E. coli*
- *S. aureus*
- Other *Enterobacteriaceae*
- *Pseudomonas aeruginosa*
- Enterococci
- Anaerobes
- *S. pneumoniae*
- Yeasts

Many other organisms have been implicated in both hospital and community-acquired bacteraemia[45-53].

Healthcare Associated Infection (HCAI)

HCAI are infections that occur as a result of healthcare interventions including care or treatment provided in the home, at the doctor's surgery or clinic, in nursing homes or following care given in a hospital. It is often difficult in patients who receive regular care to determine with accuracy whether infection is community or healthcare associated; co-operation between Public Health and Infection Control teams is therefore essential for investigative and epidemiological purposes.

Anaerobic bacteraemia

Recent studies have shown that anaerobic organisms account for 1 - 17% of positive blood cultures; anaerobic organisms are therefore still an important cause of bacteraemia and should be tested for routinely[38,54-56]. Organisms most commonly associated with anaerobic bacteraemia include[38]:

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

Bloodstream infection in children

The aetiology of paediatric bacteraemia has changed in recent years. Infections with *Haemophilus influenzae* type b have declined dramatically following the Hib immunisation programme, and systemic nosocomial infections have increased. 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, occurs less frequently[57].

Occult bacteraemia can occur in children with few or none of the symptoms normally associated with bloodstream infection[58]. Pyrexia may be the only indicator, but is non-specific. *S. pneumoniae* predominates, but occult infection with *H. influenzae*, *Salmonella* species and *N. meningitidis* has also been described.

Catheter-related bacteraemia

Confirmation that the catheter is the source of infection in intravenous catheter (IVC) related bacteraemia or fungaemia is often difficult. There is often no evidence of infection at the catheter insertion site, and the organisms involved are frequently part of the normal skin flora and are common contaminants of blood cultures.

Diagnosis of catheter related bacteraemia is usually based on[59]:

- Isolation of the same organism from the blood and purulent IVC insertion site or IVC tip
- Clinical sepsis, unresponsive to antimicrobial therapy, that resolves on catheter removal

Pregnant women

Listeria monocytogenes may cause serious infection in pregnant women. Sepsis caused by *L. monocytogenes* presents as an acute febrile illness that may affect the fetus[60,61]. 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[60].

Septic 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.

Infective endocarditis (IE)[62]

IE is infection of the heart valves and/or other areas of the endocardium. It usually occurs at the site of a predisposing cardiac lesion or congenital defect where there is turbulent blood flow, encouraging endocardial damage and adhesion of platelets[63-65]. A fibrin clot is deposited on the damaged endocardial surface and becomes colonised with organisms which have entered the bloodstream, so forming infected vegetation. Viable bacteria may be present deep within the vegetation as well as on the surface making antimicrobial treatment difficult[66].

Historically, the disease was classified as either "acute" or "subacute", relating to the usual course of the untreated disease. Proposed in 1994, the Duke criteria are now used for diagnosis[63]. It is more usual to describe the disease in relation to the infecting organism or underlying anatomy.

Native valve endocarditis

Chronic rheumatic heart disease (RHD) was the main predisposing factor in IE but has now been replaced by other conditions such as 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[66]:

- Oral streptococci
- Staphylococci (approximately 80% of these are *S. aureus*)
- Enterococci
- *Streptococcus bovis (S. bovis* biotype 1 may also be refered to as *S. gallolyticus subsp. gallolyticus)*[67]

Fungal infection is rare, except in intravenous drug users and patients with severe underlying illnesses[68]. Many other organisms have been described, including some that are fastidious, and that rarely cause human disease other than endocarditis (eg the HACEK group: *Haemophilus aphrophilus*, *Aggregatibacter actinomycetemcomitans*, *Capnocytophaga hominis*, *Eikenella corrodens* and *Kingella kingae* (see ID 12 Identification of *Haemophilus* species and the HACEK group of organisms)[63,69]. 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[69,70]. *Bartonella* species are becoming increasingly important causes of endocarditis particularly in patients with HIV infection[65].

Prosthetic valve endocarditis (PVE)

In addition to antimicrobial therapy, infected valves frequently require surgical removal and replacement either to eradicate infection or because of leakage problems. Infection may occur at any time after valve surgery, but becomes progressively less common as time passes and involves a different group of organisms. The risk of PVE in the first year is 1-5%, and after one year this decreases to about 1%[66]. The prosthetic aortic valve is more prone to infection.

"Early" PVE usually occurs within 60 days of implantation, but illness characteristic of early disease may not become apparent until 4-6 months after valve replacement. These infections reflect contamination of the valve prosthesis in the peri-operative period. Contamination usually occurs intra-operatively. "Early" PVE has a higher mortality rate than "late" PVE, and the causative organisms are often more resistant to antibiotics, probably reflecting their hospital origin and the use of prophylactic and therapeutic antibiotics peri-operatively.

The most commonly isolated organisms are[66]:

- Coagulase negative staphylococci
- *S. aureus*
- Gram negative rods
- *Candida* species
- Streptococci and enterococci
- *Corynebacterium* species

"Late" PVE may occur several years after valve implantation. The source of the infection is thought to be a transient bacteraemia or fungaemia seeding the valve as occurs in the infection of native valves, although it may be a result of delayed presentation of a hospital-acquired infection. Organisms responsible closely resemble those implicated in native valve endocarditis and include:

- Oral streptococci
- Staphylococci
- Gram negative rods
- *Candida* species
- Enterococci
- *Corynebacterium* species

Bloodstream Infection in Patients who are Immunocompromised

Patients who are immunocompromised include those with inherited, acquired or drug-induced abnormalities of the immune system. Defects in phagocytes, complement, antibody formation and cell-mediated immunity are often associated with a particular disorder or disease such as malignancy, HIV infection or sickle cell disease, and in patients who have had organ transplantation, immunosuppressive therapy or steroids[71]. The risk of infection is greatest in patients with neutropenia in whom Gram negative bacteria cause severe sepsis associated with a high mortality rate[72].

In patients who are immunocompromised there is a high incidence of infection caused by organisms that are non-virulent in the normal host and that form part of the normal host flora. These would usually be considered as contaminants in the immunocompetent host[72]. Examples are coagulase negative staphylococci, enterococci and viridans streptococci.

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[53,73].

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[53]. In addition to the organisms associated with bloodstream infection in the immunocompetent, isolates include[73]:

- Non-fermentative Gram negative rods
- *Listeria monocytogenes*
- *Corynebacterium* species
- *Candida* species

Other unusual organisms including a variety of bacteria and fungi may be isolated, many of which have very specific growth requirements[73-75].

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. However, providing bodies are kept under controlled refrigerated conditions and post mortem examination occurs within 2-10

days, it has been shown that there is no further increase in positive culture rates[76,77]. Results of post mortem blood cultures and their clinical significance should be interpreted with caution; they may however, be useful in the investigation of sudden unexpected death in infants and children (SUDI)[76-79].

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 (eg foreign travel, clinical laboratory or veterinary work posing an infection hazard) cases or clusters of the organisms below could suggest the possibility of a deliberate or accidental release of micro-organisms. Such events require a rapid response[80]; suspicion of deliberate or accidental release of micro-organisms must be notified urgently to the Public Health England 24hr Duty Doctor at Microbiology Services Colindale. The following list of organisms is not all inclusive; guidelines for managing unknown or unusual illnesses and deliberate or accidental release situations are available at: http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/DeliberateReleases

The organisms are reportable to PHE under the HPA (Notification) Regulations 2010; a comprehensive list of causative agents notifiable to the Health Protection Agency is available at: http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/NotificationsOfInfectiousDiseases/ListOfCausativeAgents/

If the following organisms are suspected, investigation should be carried out at containment level 3 unless otherwise stated. Suspect isolates should be sent to the appropriate reference laboratory for characterisation.

- *Bacillus anthracis* (Anthrax)
- *Brucella* species (Brucella)
- *Francisella tularensis* (Tularaemia)
- *Burkholderia mallei* (Glanders)
- *Burkholderia pseudomallei* (Melioidosis)
- *Clostridium botulinum* (Botulism)

  May be investigated at Containment level 2 in a Microbiological Safety Cabinet

  Refer to ID 8 Identification of *Clostridium* species
- *Coxiella burnetii* (Q fever)
- *Yersinia pestis* (Plague)

Note: *Brucella* species, *B. mallei, B. pseudomallei* and *Y. pestis* are listed in the databases of a number of commercially available kit-based identification systems; results should however be interpreted with caution.

Note: *B. anthracis, Brucella* species, *C. botulinum* and *Y. pestis* all cause disease which is reportable to the Local Authority Proper Officer under the Health Protection (Notification) Regulations 2010. A comprehensive list of diseases notifiable to the Local Authority Proper Office under the Health Protection (Notification) Regulations 2010 is available at:

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/NotificationsOfInfectiousDiseases/ListOfNotifiableDiseases/

Note: Brucellosis is reportable under the Zoonosis Order 1989.

Increasing Antibiotic Resistance[81,82]

Antibiotic resistance especially amongst Gram negative bacteria has increased markedly in the past 15 years[79]. Prior to this, Gram negative bacteria were, in general, sensitive to aminoglycosides, third generation cephalosporins and fluorinated quinolones. However, resistance mechanisms have evolved to not just one, but several classes of antibiotic simultaneously[79]. Of concern are extended spectrum β-lactamase producing (ESBL) Enterobacteriaceae, carbapenem resistant *Enterobacteriaceae* and multidrug resistant *Pseudomonas aeruginosa*[81,83]. In 2008 just under 24,000 *E. coli* bacteraemia cases were reported nationally and of these 20% were resistant to quinolones, 9% to third generation cephalosporins and approximately 7% to gentamicin[79]. The incidence of multidrug resistance in Gram positive organisms such as *S. aureus*, coagulase negative staphylococci and enterococci has also increased in recent years[81]. The net result is an increasing number of patients for whom initial empirical antibiotic therapy is ineffective[25].

The prevalence of multi drug resistant Gram negative bacteria, meticillin resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE) and other resistant organisms highlights the need for accurate and timely blood culture results to ensure correct antibiotic treatment is being administered and to reduce the overall use of broad spectrum antibiotics[81,84,85].

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, thereby generating the best outcomes.

The introduction of commercial, fully automated, continuous-monitoring blood culture systems has led to earlier detection and better identification of pathogens. This is particularly true of organisms considered most pathogenic, for example *S. aureus*, Gram negative rods and streptococci[14]. However, blood culture does have its limitations.

Pre-analytical[6]

The pre analytical stage from collection to loading is dependent on many factors:

- The location of the Laboratory in relation to the ward (Onsite/Offsite)
  - External transportation arrangements (frequency, out of hours service etc)
  - Internal transfer arrangements (frequency, availability of pneumatic tube transport, out of hours service etc)
- Level of Laboratory out of hours service provision (out of hours loading frequency etc)
- Equipment available and developments in current technology (availability of continuous monitoring blood culture system, pre incubation incubator etc)

With continuous monitoring blood culture machines, blood cultures should ideally be placed on the machine 24hr a day, as soon as possible after collection and within a maximum of 4hr.

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 a percentage of positive cultures may not be detected once placed on the blood culture machine after pre incubation (see Technical Information/Limitations). Consequently many laboratories do not now pre-incubate blood cultures leaving them at room temperature overnight, leading to an increased time to detection (time from loading to flagging positive) once placed on the machine (see Technical Information/Limitations/Appendix 1). A balance between false negative blood culture and incurring significant delay before a Gram stain result can be obtained needs to be achieved.

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[6]:

- Consider external and internal transport arrangements
  (decrease collection to loading time)
- Consider shift working patterns or out of hours laboratory cover
  (decrease collection to loading time)
- Consider use of non-Microbiology (eg Blood Sciences) personnel to load machines out of hours
- Consider use of equipment
  For example controlling the time of operation of pre-incubators using a seven day programmable time switch (in this instance all blood cultures received outside of normal working hours would be loaded in the incubator overnight but this would only be powered for a limited number of hours for example midnight to 9am)
  (decrease time to positivity)
- Consider new developments/advancements in current technology
  (decrease collection to loading/time to positivity)

Analytical

The time to detection (TTD) once samples are loaded is dependent on the time required for multiplication to a significant level to occur; fastidious or non-cultureable organisms may fail to grow and sensitivity may be decreased when samples are taken directly after antibiotic treatment[2].

Blood culture systems should therefore aim to achieve the following:

- A culture medium as rich as possible to allow the recovery of very small numbers of a variety of fastidious organisms
- Neutralisation or removal of antimicrobial substances, either natural blood components or antimicrobial agents
- Minimisation of contamination
- Earliest possible detection of bacteria and fungi

Blood culture systems rely on a variety of detection principles and cultural environments to detect micro-organisms. Many systems and their respective media have been compared, each system having its own limitations and advantages[14,86-91]. Fully automated continuous monitoring systems are simple to use in comparison with manual and semi-automated systems.

Most systems employ both aerobic and anaerobic bottles for adults, but provide a single aerobic bottle for use with children for whom blood specimen volumes obtained are often small[92].

Factors Affecting Isolation of Causative Organisms

A number of clinical and technical factors may affect the isolation of the infecting organism, regardless of the system employed[14,53].

Clinical:

Method of Collection

Collection of blood from the patient should be carried out following Department of Health guidance[93].

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[94,95]. 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[96]. 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[97-99].

Number and Timing of Samples

For the majority of patients, two 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[100]. In most conditions other than endocarditis, bacteraemia is intermittent, being related to the fevers and rigors which occur 30-60 minutes after the entry of organisms to the bloodstream. Samples should be taken as soon as possible after a spike of fever. However, some work has shown little difference in isolation rates between blood drawn at intervals and simultaneously with fever spikes[101]. Certainly, the timing is less important for continuous bacteraemia, as seen in infective endocarditis.

Previous Antimicrobial Therapy[2]

Ideally, blood samples should be taken prior to antimicrobial treatment. When already receiving antimicrobials, blood culture should be collected just before the next dose is due when antimicrobial concentration in the blood is at the lowest. Any recent antimicrobial therapy can have a significant effect on blood culture results by decreasing the sensitivity of the test. This may be of particular importance in those patients receiving prophylactic antibiotics and who are at high risk of bloodstream infections. If patients have received previous antimicrobial treatment, bacteraemia should be considered even if blood culture results are negative.

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, with approximately a 3% increase in yield per mL of blood cultured. False negatives may occur if inadequate blood culture volumes are submitted[102].

The number of organisms present in adult bacteraemia is frequently low, often $<1 \times 10^3$ colony forming units per litre (cfu/L)[103]. For adult patients it is recommended that 20-30mL of blood be cultured per set[55,104]. Most modern commercial systems allow 10mL blood to be added to each bottle. Manufacturers' optimum blood volume recommendations vary; manufacturers' instructions should be read prior to use.

Data regarding the optimum total blood volume per set for neonates and children is limited. The criteria for calculating total blood culture volumes is often based on weight rather than age and relates to total patient blood volume[102]. 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[105,106].

Low level bacteraemia (<4 x 10^3cfu/L) in neonates and children does occur with clinically significant organisms, one study suggests that for the reliable detection of low level bacteraemia, 4 to 4.5% of a patient's total blood volume, not 1%, should be cultured[107].

Technical:

Media Used

Most systems employ different media for the isolation of aerobic and anaerobic organisms, and some media are specifically designed for the detection of organisms such as fungi and *Mycobacterium* species. A variety of blood culture media and systems are commercially available which have been evaluated[108-111]. 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[112,113]. 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*[14,114-116].

A blood to broth ratio of about 1:15 is required to remove the antibacterial effects of normal human blood, this may be reduced to between 1:5 and 1:10 by the addition of 0.05% sodium polyanethol sulphonate (SPS)[14,55,100]. Failure to keep to this ratio may result in false negative culture. SPS has an inhibitory effect on *Neisseria* species, anaerobic cocci, *Streptobacillus moniliformis* and *Mycoplasma hominis*[117]. The inhibitory effects of SPS may be reduced by the addition of gelatin to the broth[118,119]. The medium in some commercially available bottles is supplemented with materials which improve microbial recovery by adsorbing antimicrobial substances and which lyse WBCs to release organisms into the blood broth mixture[14].

Neutralisation of Antimicrobial Agents

At the time of blood culture sampling 28-63% of patients are in the process of receiving antimicrobial treatment which may have a negative effect on organism recovery[110]. Media containing antibiotic inactivating resins and other adsorptive materials including charcoal have been developed to overcome the effect of antimicrobials[110,111]. Some media, however, rely on optimal blood-broth dilution for antimicrobial neutralisation[111]. Lysis-centrifugation techniques have been used, but there are conflicting reports concerning both their efficacy and the clinical importance of the increased isolation rates attributed to them[120-123].

Incubation Time and Temperature

A temperature of 35-37°C for 5-7 days is recommended for routine blood cultures[14]. Five days is usually sufficient incubation time for the recovery of most organisms if automated systems are used[106]. If conditions such as brucellosis are suspected, 2- 5 days incubation is usually sufficient, however, the incubation period may need to be extended to 10 days depending on media used, and a terminal subculture may be required[17-19]. It is advisable that if these bacteria are suspected that all culture is suspended and the samples sent to the reference laboratory.

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[124]. The increased yield may be small for some organisms (HACEK) and specialised methods rather that extended incubation times may be more likely to improve recovery[14,69,70,125].

Agitation of Media

The effects of agitation are usually an increased yield and earlier recovery for aerobic bottles; agitation of anaerobic bottles does not increase yield, and agitation of mycobacterial blood cultures decreases yield[126,127]. Continuous monitoring systems incorporate a variety of types

and speeds of agitation, and the semi-automated systems include an initial period of agitation for the aerobic bottles. Agitation of the aerobic bottle should be considered in conventional manual systems.

Headspace Atmosphere

Headspace atmosphere will depend on the system used, but may influence the rate of growth of some organisms. The headspace of aerobic bottles usually contains air with various concentrations of CO_2 and may require venting to increase the O_2 content. Depending on the system, the headspace of anaerobic bottles usually contains combinations of CO_2 and nitrogen.

Subculture

If manual or semi-automated systems are used, subculture of both bottles in a set where only one bottle flags positive reveals both to be positive in about 50% of cases. It is probably unnecessary to subculture both for continuous monitoring systems. Subculture of anaerobic bottles via a sub-vent unit, loop or pipette will allow air into the headspace unless performed in an anaerobic cabinet and may adversely affect subsequent growth of anaerobic organisms. Diphasic systems have the advantage of simple closed subculture, achieved by tilting of the bottle, but colony recognition may be impaired by the glass[14].

Blind or terminal subculture

Blind or terminal subculture is not routinely recommended for blood cultures if automated systems are used (manufacturers' instructions should be followed), but may be indicated for manual systems[16]. Some organisms such as *Neisseria* species, *Brucella* species, *Francisella* species, *H. influenzae* and *Legionella* species may give weak signals or may be present in blood culture media without showing visible signs of growth. Similar effects have been reported for *P. aeruginosa* and *Candida* species[128]. Blind subculture (at appropriate containment level) of bottles from patients where clinical presentation or history is indicative of such organisms may be considered.

Rapid Identification and Direct Sensitivity Testing

Following conventional practice identification and sensitivities of resistant organisms may not be available until 24-48hr post flagging positive; important information may therefore be significantly delayed, causing further delay in specific pathogen directed antimicrobial treatment[7]. Using rapid tests it is possible for identification and susceptibility results to be available within 24hr of flagging positive.

To reduce turnaround times, rapid identification and sensitivity tests should be performed in conjunction with routine methods where appropriate. A variety of rapid identification and sensitivity methods have been evaluated; these include tube coagulase, antigen latex, molecular techniques and the recently developed Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)[129,130]. It is important to ensure that fresh cultures of pure single isolates are tested to avoid reporting misleading results.

Laboratories should follow manufacturers' instructions and all rapid tests must be validated and be shown to be fit for purpose prior to use.

Rapid Identification

Antigen Agglutination Test

Antigen agglutination tests are used to test an unknown organism against known antisera. They are used for example in the serotyping of *Salmonella* species and the grouping of streptococci[131,132]. Lancefield grouping of streptococci direct from culture is useful as

grouping is clinically significant and may affect antimicrobial treatment. Antigen testing of blood culture samples is also useful in confirming the presence of *S. pneumoniae* that has undergone autolysis. See TP 3 – Agglutination Test.

Coagulase Test

Members of the genus staphylococcus are differentiated by the ability to clot plasma by the action of the enzyme coagulase. Rapid tests which differentiate between coagulase positive (including *Staphylococcus aureus*) and coagulase negative staphylococci are well documented[133-135]. Tube coagulase, agglutination, conventional PCR techniques and molecular techniques with fluorescent labelled probes have also been shown to identify coagulase positive staphylococcus direct from blood culture. Variable sensitivities and specificities have been reported, and may be medium dependent[14,136]. See TP 10 – Coagulase Test.

Automated Identification Methods

There are several automated systems available which are capable of performing identification (and sensitivity testing) on positive isolates using microtitre broth dilution techniques[8,9,137]. Comparative studies have shown that results from automated systems tested are reliable (particularly for Gram negative organisms) and can provide results in half the time required for conventional methods[138].

Molecular Methods[82]

There is growing interest in the use of Polymerase Chain Reaction (PCR) tests and other nucleic acid amplification techniques (NAATs) for identification of bacteria from positive blood samples[10]. PCR targets conserved genes on the bacterial genome and enables the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short time particularly if multiplex real-time PCR is used[139]. Several assays are available including pathogen specific assays (designed to detect one target in a positive blood sample), broad range assays (using primers that recognise conserved sequences encoding pathogen ribosomal DNA) and multiplex assays (designed to detect the most frequent pathogens in a single reaction) followed by genus or species identification[11].

MALDI-TOF Mass Spectroscopy

Recent developments in identification of bacteria, yeast and fungi include the use of 16s ribosomal protein profiles obtained by Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-TOF) mass spectroscopy[139]. Mass peaks achieved by the test strains are compared to those of known reference strains. It is possible for an organism to be identified from an isolate within a short time frame and it is increasingly being used in laboratories to provide a robust identification system. The use of MALDI-TOF-MS in the identification of organisms directly from positive blood culture is currently being evaluated[12,139-143]. Studies have shown that direct identification of Gram positive bacteria (particularly staphylococci) is less reliable than Gram negative bacteria and that media composition (eg inclusion of charcoal) may affect identification[12,139,141-143]. Other studies have shown that rapid identification using MALDI-TOF leads to a decrease in the time to identification and also results in an increase in the proportion of patients on appropriate antimicrobial treatment[140].

Direct Sensitivity

To improve the quality of sensitivity testing there has been a general movement away from performing direct sensitivities on clinical samples, although the British Society for Antimicrobial Chemotherapy (BSAC) does however recognise that the procedure is carried out in many laboratories as a means of providing rapid results[144]. To reduce turnaround times, it is recommended that direct sensitivity tests are performed on positive blood culture bottles where appropriate, recognising that sometimes different organisms may be identified from

different bottles within a pair. Results should be interpreted with care, especially if the inoculum is lighter or heavier than the recommended semi-confluent growth.

Antibiotic Disc Diffusion Method

Direct disc diffusion is not a novel method, but is rapid, easy to perform and inexpensive[145]. High rates of disparity have however been shown when comparing the disc method to automated methods[9].

Minimal Inhibitory Concentration (MIC) Tests

Broth and agar dilution methods can be used to determine the lowest concentration of an antimicrobial agent able to inhibit growth under test conditions. The MIC value can be used to determine antimicrobial susceptibility of a specific strain against a particular antimicrobial drug. Antibiotic gradient strips which evaluate MIC have also recently been developed and may be used to acquire rapid results[146].

Rapid results obtained by such means may influence patient management, improve laboratory work-flow and reduce costs. It is important that results of identification and sensitivity testing of blood cultures using commercial or other products should be viewed with caution unless they have been validated. Where the culture is mixed or the inoculum level is incorrect sensitivity tests should be repeated[144].

Contamination

Contamination of blood cultures complicates interpretation and can lead to unnecessary antimicrobial therapy and increased costs. In general, contamination target rates are set at less than 3%[93,147,148]. 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, time to positivity, quantity of growth, and clinical and laboratory data (including source of culture)[149,150]. Prevention of contamination can be achieved through appropriate skin and bottle preparation, obtaining cultures from peripheral venepuncture instead of via vascular catheters, and through training and intervention measures[147,150,151].

Technical Information/Limitations

Specimen Containers[2,152,153]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Pre-Incubation of Blood Cultures

The recognition that certain non-fermenting, Gram negative bacteria such as *Pseudomonas aeruginosa*, *Streptococcus* species and yeasts may not be detected in continuous monitoring blood culture systems if pre-incubated at 35-37°C has had a significant effect on laboratory practice, resulting in many laboratories storing and transporting delayed samples at room temperature[2,154,155].

These organisms may fail to trip the threshold algorithm of the continuous monitoring blood culture machine. Detection of their presence in positive blood cultures is dependent upon biochemical changes during the growth phase. When pre-incubation has been sufficiently

long for the organism to have gone through the growth phase and be in the stationary or decline phase, bottles containing such organisms will not register positive.

It is estimated that 2-5% of positives 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 or TTP) for many organisms may be doubled or tripled[2,4,5,32].

Laboratories should investigate peer reviewed literature and clinical laboratory textbooks and validate methods used if not following the manufacturers' instructions.

All delayed cultures should be inspected for signs of growth including yellowing of the sensor, haemolysis, gas production or turbidity. If microbial growth is confirmed by Gram stain, the bottle should be treated as positive and subcultured as appropriate.

Inconsistent Results

Positive appearance/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[65,156,157]. 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*[158]. 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 or consider direct antigen testing by a validated method on the broth bottle.

Positive appearance/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's or Sandiford's may be required to demonstrate the presence and morphology of the organisms involved[159]. This may give guidance for the selection of appropriate media for subcultures.

Negative appearance/negative flag with positive Gram stained film and positive subculture.

Refer to section on subculture on page 22.

1 Specimen Collection, Transport and Storage[152,153]

1.1 Safety Considerations[160-171]

Use aseptic technique.

Inspect the blood culture bottles for damage.

Ensure that the blood culture bottles have not exceeded their expiry date.

Do not re-sheathe needles.

Collect specimens in appropriate CE marked leak proof containers (according to manufacturers' instruction if using a continuous monitoring blood culture system).

Compliance with postal and transport regulations is essential.

1.2 Achieving Optimal Conditions

Collect specimens as soon as possible after the onset of clinical symptoms. Drawing blood before or as soon as possible after a fever spike is optimal, except in endocarditis where timing is less important[101].

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[3].

1.2.1 Time between specimen collection and processing

Collect specimens before antimicrobial therapy where possible[172].

Inoculated bottles should be loaded to continuous monitoring blood culture systems as soon as possible, and within a maximum of 4 hours[4-6].

1.2.2 Special considerations to minimise deterioration

Samples should not be refrigerated[172].

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[154,155].

Automated systems

In order to minimise the risk of autolysis of certain organisms such as *S. pneumoniae*, bottles should be subcultured as soon as possible after a positive flag is detected[158].

1.3 Correct Specimen Type and Method of Collection

Sampling of blood should be carried out according to Department of Health guidance[93].

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. If necrotising enterocolitis is suspected and sufficient blood is obtained, inoculate a 'low volume' and an anaerobic bottle.

Appropriate blood culture bottles must be used for specific machines when using continuous monitoring blood culture systems and manufacturers' instructions should be followed.

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 septum's integrity.

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 or ESR 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.

1.4 Adequate Quantity and Appropriate Number of Specimens

Blood culture is a culture of blood collected from a single venepuncture site inoculated to one or multiple bottles.

A blood culture set is defined as one aerobic and one anaerobic bottle. For infants and neonates, a single aerobic bottle may be requested.

Quantity

Adults

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

Note: More than 2 bottles per set may be indicated.

Children and neonates

No more than 1% of total blood volume.

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 inadequate for two 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 two consecutive sets from two separate venepuncture sites during any 24hr period for each septic episode[100]. For neonates take a single aerobic bottle or special low volume bottle.

Take two sets during the first hour in cases of severe sepsis prior to commencing antibiotic treatment, provided this does not significantly delay antibiotic administration[30].

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

2 Specimen Processing[152,153]

2.1 Safety Considerations[160-171]

Containment Level 2.

All specimens should be processed at Containment Level 2 unless infection with a Hazard Group 3 organism (eg *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)[163]. Ideally all blood cultures should be subcultured in a MSC because clinical details may be lacking and may not highlight the possibility of Hazard Group 3 organisms.

N. meningitidis causes severe and sometimes fatal disease. Laboratory acquired infections have been reported. The organism infects primarily by the respiratory route. An effective vaccine is available for some meningococcal groups.

N. meningitidis is a Hazard group 2 organism and the processing of diagnostic samples can be carried out at Containment Level 2.

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 28-30°C. Care should be taken with yeast isolates if there is a relevant travel history especially in HIV-infected individuals.

Avoid the use of sharp objects 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 SMI.

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

2.2 Test Selection

Incubate the bottles at 35-37°C for 5-7 days.

2.3 Appearance

Inspect the bottles visually for evidence of microbial growth.

2.4 Microscopy

Positive bottles - all systems

Perform microscopy on broth from any bottle which "flags" positive or which is visually positive (bowed septum, bloody/lysed or indicator colour change).

If using a diphasic medium, prepare a Gram stained film from both the buffy layer and the agar surface.

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
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's or carbol fuchsin counterstain is used[159] (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 2.5.3) 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.

2.5 Culture and Investigation

2.5.1 Pre-treatment

N/A

2.5.2 Specimen processing

Standard

Positive bottles from all systems

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 one drop on to each agar plate.

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.

Positive bottles from manual systems

Subculture all bottles of the set as described above, even if only one 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 - 48hr[173].

Supplementary

Flag/appearance positive, but culture negative - 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 2.5.3. Refer to Technical Information/Limitations for further information.

2.5.3 Media for subculture, conditions and organisms

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
All clinical conditions	Blood agar†	35-37	5-10% CO2	40-48hr*	Daily	Any organism may be significant
	Fastidious anaerobe agar	35-37	anaerobic	40-48hr*	≥40hr and up to 5d	Any organism may be significant
For these situations add the following:						
Clinical details/ conditions	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	Chocolate agar†	35-37	5-10% CO2	40-48hr	Daily	*Haemophilus* species *N. meningitidis* *N. gonorrhoeae*
Gram negative rods seen on microscopy	MacConkey/ CLED agar or Chromogenic agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae Non–fermentative organism *Pseudomonas* species
Microscopy suggestive of mixed or anaerobic infection	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	5-7d	≥40hr and at 5d	Anaerobes
Systemic fungal infection#	Sabouraud's agar	28-30	air	5d	2d and at 5d	Yeast Mould

Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Primary culture negative and positive growth curve‡ (subculture all bottles)	Blood agar	35-37	micro-aerobic	5d	≥3d and at 5d	*Campylobacter* species *Helicobacter* species
	Blood agar with streak of *S. aureus* (NCTC 6571)	35-37	5-10% CO2	40-48hr	≥40hr	*Abiotrophia* species
	Fastidious anaerobe agar	35-37	anaerobic	5d	≥40hr and at 5d	Cysteine-dependent organisms
	MacConkey/ CLED agar	35-37	air	16-24hr	≥16hr	

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 ≥40 hours and left in the incubator/cabinet for up to 5 days.

#where clinically indicated, blood culture bottles may require an extended incubation of up to three weeks for *Cryptococcus* species and up to six weeks for *Histoplasma* species[21,174-176].

‡other organisms may need to be considered.

Rapid tests such as antigen detection, PCR should be performed according to manufacturers' instructions.

2.6 Identification

Refer to individual 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 one 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 (eg typing isolates from nosocomial infection).

2.7 Antimicrobial Susceptibility Testing

To reduce turnaround times, it is recommended that direct sensitivity tests are performed on all positive blood culture isolates where appropriate.

Refer to British Society for Antimicrobial Chemotherapy (BSAC) guidelines[144].

2.8 Referral to Reference Laboratories

Contact appropriate reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission. Information regarding specialist and reference laboratories is available via the following websites:

HPA - Specialist and Reference Microbiology Tests and Services

Health Protection Scotland – Reference Laboratories

Belfast Health and Social Care Trust – Laboratory and Mortuary Services

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.

2.9 Referral for Outbreak Investigations

N/A

3 Reporting Procedure

3.1 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).

3.1.1 Microscopy reporting time[12]

Results should be communicated immediately, within a two hour period. Written or computer generated reports should follow preliminary/verbal reports within 24hr.

3.2 Culture

Following results should be reported:

- All organisms which are isolated (with comment if isolate of doubtful significance)
- Absence of growth
- Results of supplementary investigations

3.2.1 Culture reporting time

Preliminary positive culture reports should be telephoned or sent electronically stating, if appropriate, that a further report will be issued. Final written or computer generated reports should follow preliminary/verbal reports on the same day as confirmation where possible, and within a maximum of 24hr[25].

Preliminary negative results should be reported at 36hr from collection for neonates and 48hr for all other patients (or as per local agreement). It is anticipated that preliminary negative reports will be generated automatically to closely reflect the true incubation time. Final reports should be generated within five days of receipt 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 48hr after the preliminary report.

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

3.3 Antimicrobial Susceptibility Testing

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

4 Notification to PHE[177,178]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify PHE when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAIs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland[179] and Wales[180].

A comprehensive list of causative agents notifiable to the Public Health England under the HPA (Notification) Regulation 2010 is available at: http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/NotificationsOfInfectiousDiseases/ListOfCausativeAgents/.

Appendix 1: Critical Control Points in Blood Culture Investigation

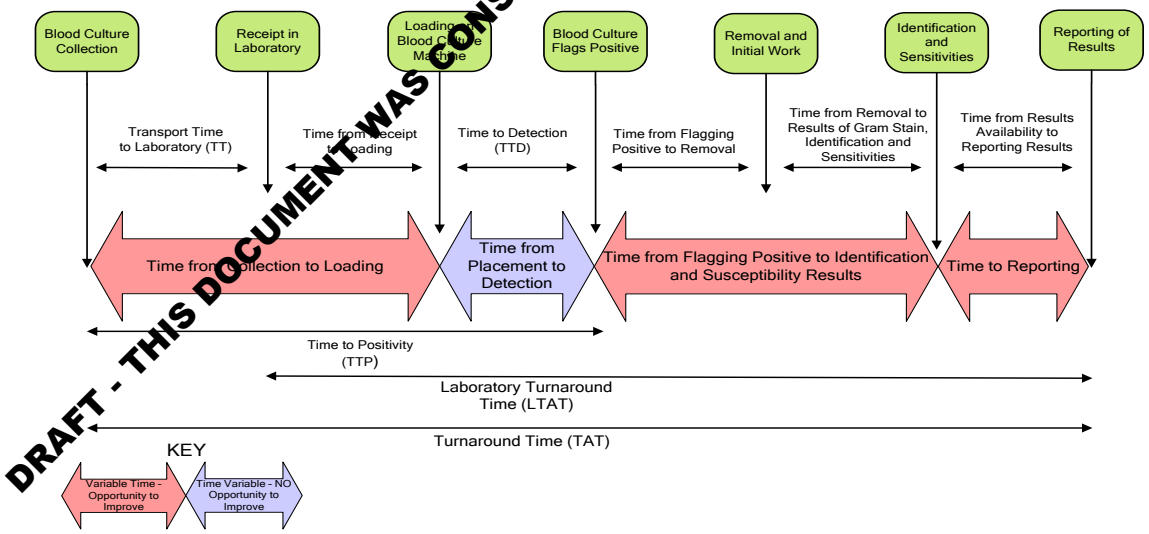
By breaking down the blood culture process it is possible to identify critical control points where there may be delays or the potential to improve turnaround times (TAT)[2].

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[181] - Time to Detection (TTD)
- Time from flagging positive to identification and susceptibility results[25]
- Time from identification and susceptibility results to reporting

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 two 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.

Timeline - Critical Control Points



Decreasing TAT leads 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[32].

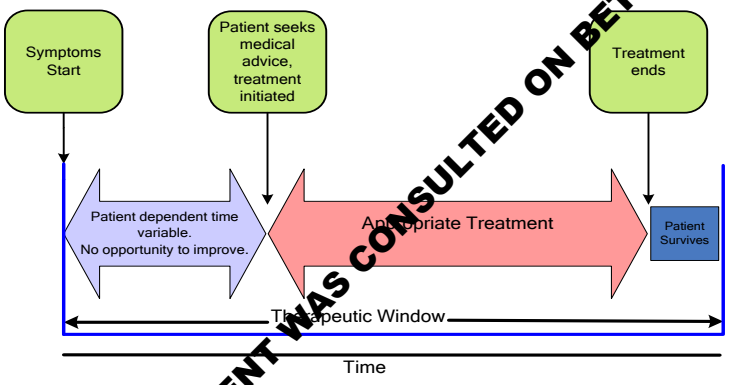
Appendix 2: Therapeutic Window

For each patient there is a period of time within which the infection and the patient can both be successfully treated, this can be thought of as the 'Therapeutic Window'. There comes a point outside the window period at which, even though the infection may be brought under control or eradicated, the patient will not survive as the resultant inflammatory cascades or organ damage has gone beyond a stage at which it is reversible[85]. The aim is therefore to deliver appropriate therapy including antibiotics within the window period. The size of the therapeutic window varies enormously and may be very short or indefinitely long dependent on the organisms and patient involved. The optimal approach involves early prescription of broad-spectrum antibiotics followed by timely responses to both microbiological and clinical results as and when they become available[34].

The following four scenarios demonstrate the potential influence of blood culture results on patient outcome[27]:

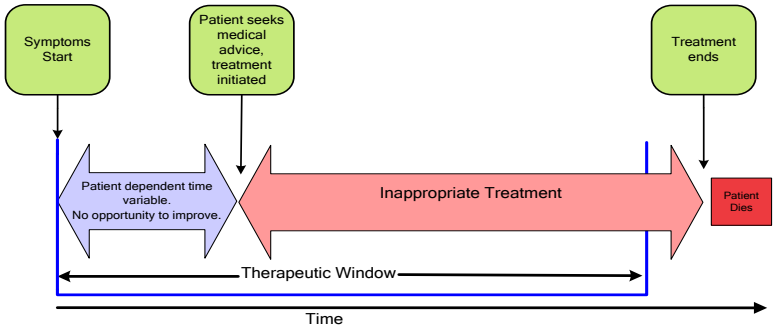
1. **Appropriate treatment is received within the therapeutic window and the patient survives.**

   The therapeutic window outlines a period of time within which both the infection and the patient can be successfully treated. The size of the therapeutic window varies enormously. For example in a young patient with cystitis the window may appear indefinitely long. At the other extreme, in the very septic patient, the therapeutic window may be very short. Any delay beyond this in initiating appropriate treatment is associated with an adverse outcome. Sepsis pathways recognise the importance of prompt antibiotic therapy including this as a key action.



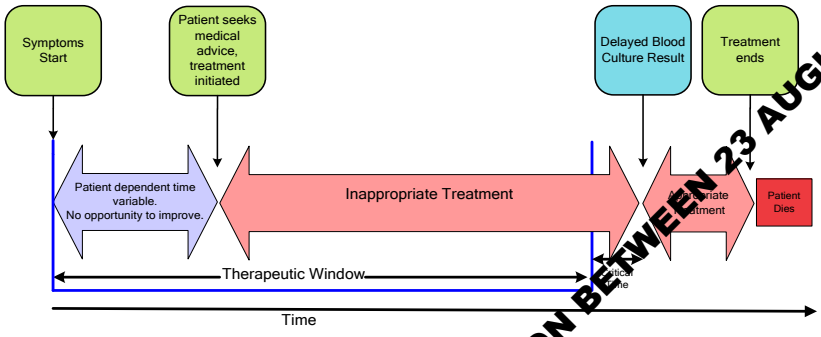
2. **The patient receives inappropriate empirical antibiotic throughout the therapeutic window and does not survive.**

   The duration of the therapeutic window may in this instance have been very short extending up to several days. Sepsis evolves over a period of time. Thus when the patient was first seen their condition may have been stable with a relatively long therapeutic window. Incorrect antibiotics were prescribed and time is lost as it is difficult to judge their effectiveness in the first 24-48 hours. For a variety of reasons the appropriate treatment is not delivered and the patient dies.



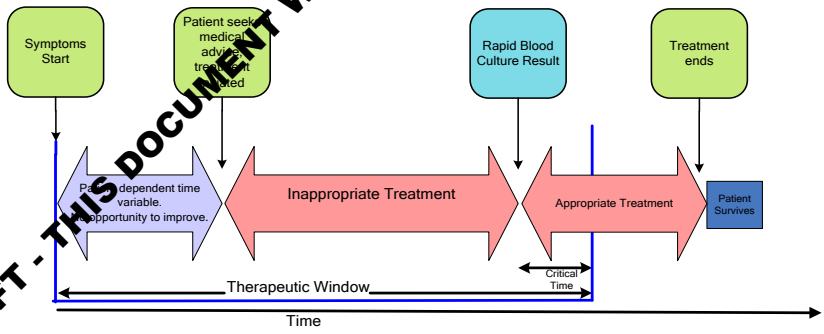
3. The patient receives appropriate treatment outside of the therapeutic window and does not survive.

   The results of a blood culture identify that the patient is receiving inappropriate treatment. However, the blood culture result is received too late, falling outside the 'window of opportunity'. Even though appropriate treatment is initiated the patient's fate has already been sealed. Whereas the bacterial infection may be eradicated by antibiotics the effect of the infection on the patient has become irreversible.



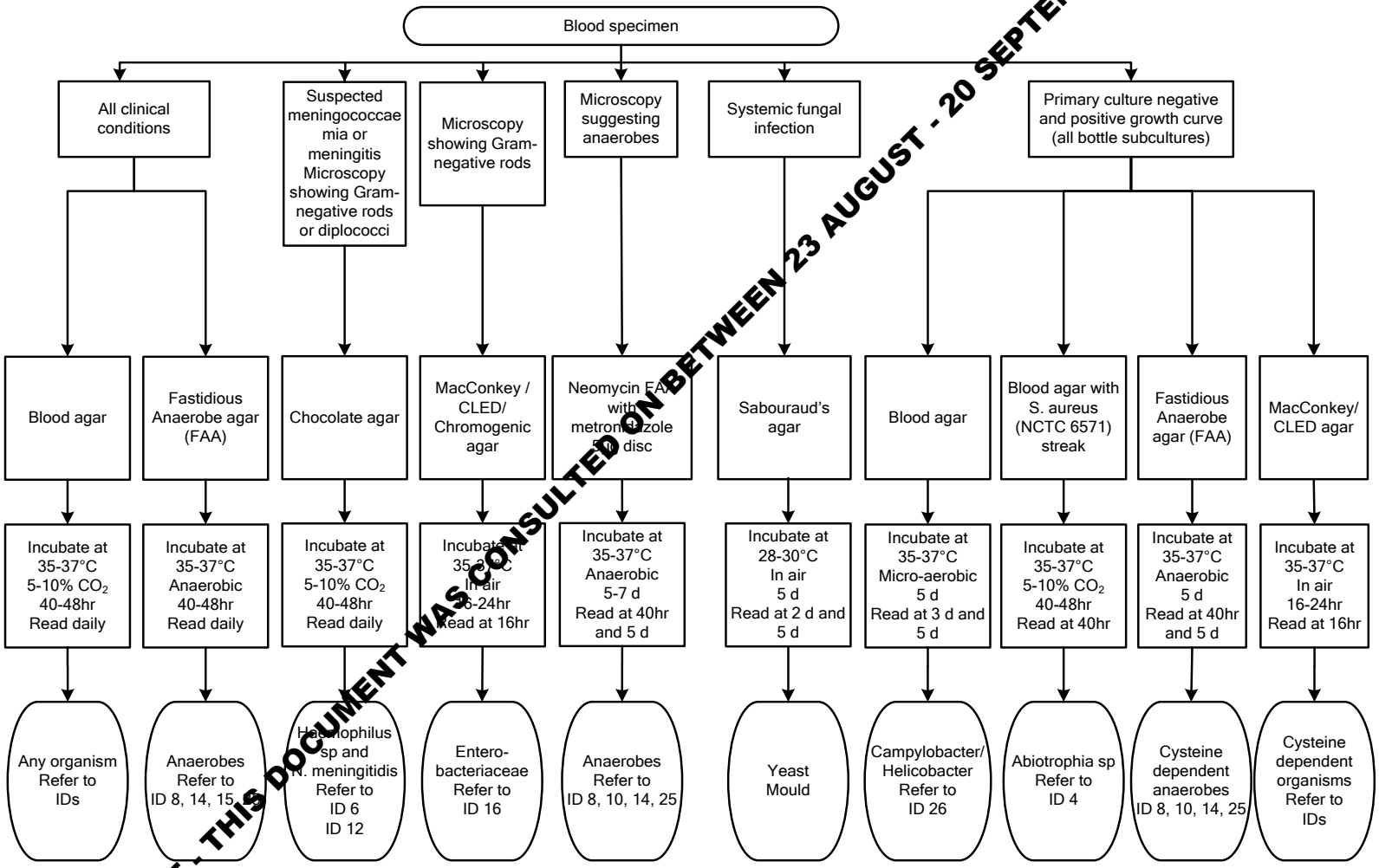
4. The patient receives appropriate treatment within the therapeutic window and survives.

   The blood culture result has been received within the therapeutic window resulting in institution of appropriate therapy and a successful outcome. The patient's clinical condition was stable on admission but deteriorated with incorrect treatment rapidly approaching the end of the therapeutic window.



In scenarios three and four 'critical time' periods are illustrated, the time between the end of the window and the blood culture result being available. The critical time period is highly variable, but it is known that it could be very short for some patients. In contrast processing a blood culture rapidly (scenario four) can expedite a result by 24 hours or more, easily enough to shift the administration of appropriate therapy back within the window period for some patients.

Appendix 3: Investigation of Blood Cultures (for Organisms other than *Mycobacterium* species) Flowchart



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