

NATIONAL STANDARD METHOD

**INVESTIGATION OF
BLOOD CULTURES
(FOR ORGANISMS OTHER THAN
MYCOBACTERIUM SPECIES)**

BSOP 37

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



Association of Medical Microbiologists & Immunologists



INVESTIGATION OF BLOOD CULTURES (FOR ORGANISMS OTHER THAN *MYCOBACTERIUM* SPECIES)

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AMENDMENT PROCEDURE

Controlled document reference	BSOP 37
Controlled document title	Investigation of blood cultures (for organisms other than <i>mycobacterium</i> species)

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
6/ 09.08.05	4.1	5			

UNDER CONSULTATION

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Type of specimen: Blood culture

Other specimens may be processed in blood culture bottles where appropriate see [BSOP 26 – Investigation of fluids from normally sterile sites](#).

SCOPE OF DOCUMENT

This NSM describes the processing and microbiological investigation of blood cultures. It does not address the detection of parasites, viruses, organisms associated with bioterrorism and deliberate release or of *Mycobacterium* species (see [BSOP 40 – Investigation of specimens for *Mycobacterium* species](#)) or list specific details of commercially available systems.

INTRODUCTION

The culture of micro-organisms from blood is essential in the laboratory diagnosis of bacteraemia, infective endocarditis and many infective conditions associated with a clinical presentation of pyrexia of unknown origin (PUO). 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 bacteraemia in association with other infectious diseases such as septic arthritis and pneumonia. Early positive results provide valuable diagnostic information on which appropriate antimicrobial therapy can be based, so helping to reduce mortality².

The blood stream contains many antimicrobial components including lysozyme, leucocytes, immunoglobulins and complement. Bacteria may enter the bloodstream from a focus of infection within the body, from a surface site colonised with normal flora through broken skin or mucous membrane, from the gastrointestinal tract or by the direct introduction of contaminated material into the vascular system^{2,3}. These bacteria are normally removed from the bloodstream within a few minutes, and 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 disease⁴⁻⁶.

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 listed below could suggest the possibility of deliberate release of micro-organisms. Suspicion of deliberate release of micro-organisms must be notified urgently to the Consultant for Communicable Disease Control and the Communicable Disease Surveillance Centre:

- *Bacillus anthracis* (causative organism of anthrax)
- *Brucella* species (causative organism of brucellosis – syn. “undulant fever”; “Malta fever “ “Mediterranean fever”)
- *Burkholderia mallei* (causative organism of glanders)
- *Burkholderia pseudomallei* (causative organism of melioidosis – syn. “pneumoenteritis”)
- *Yersinia pestis* (causative organism of plague)
- *Francisella tularensis* (causative organism of tularaemia – syn. “deerfly fever”, “rabbit fever”)

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All of the above organisms are potentially dangerous to handle in the clinical laboratory, especially *Brucella* species, *F. tularensis*, and *B. mallei*. Pathogenic *Burkholderia* species may be especially dangerous to laboratory workers with states of altered or impaired immunity, including diabetes mellitus.

Note: Anthrax and plague are statutorily notifiable to the Local Authority and brucellosis is Reportable under the Zoonosis Order 1989.

Bacteraemia The occurrence of organisms in the blood. It may be transient, intermittent or continuous⁷.

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

Intermittent bacteraemia is really "recurrent transient" bacteraemia 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 the intermittent bacteraemia as bacteria tend to be cleared by host defence mechanisms before then.

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.

Two sets of blood cultures taken over an undefined period (in practice over several hours or days) will increase the sensitivity of detection⁸⁻¹¹. It is rarely justified to take more than two sets as sensitivity is not increased any further. An exception to this rule would be endocarditis where the previous bottles had been shown to be negative. In this case it may be worth while taking up to four sets of blood cultures¹².

Septicaemia is difficult to define. It is often used interchangeably with bacteraemia and sepsis and usually implies the presence of clinical symptoms or on whether or not the bacteria are considered to be multiplying in the blood, but this cannot be verified^{3,4}.

Symptoms are often non-specific and diagnosis is frequently based on a high degree of clinical suspicion. Symptoms may include fever, chills and hypotension or the patient may experience a more overwhelming illness with toxæmia, acidosis, rigors and multi-organ failure. Fever does not present in all patients with infection, notably the elderly¹¹, and temperature is lower than 38°C in some patients^{2,13}. Fever may also be modified or eradicated by antipyretics such as paracetamol.

Sepsis is the term used to describe the state where there is clinical evidence of infection as well as a systemic response, manifested by fever, tachycardia and tachypnoea⁴.

Sepsis syndrome is defined as sepsis plus evidence of abnormal perfusion of any organ, with at least one of the following: elevated blood lactate, oliguria, altered mental state or arterial hypoxia⁴.

Septic shock occurs in patients with sepsis syndrome and hypotension.

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Pathogenesis of the Sepsis syndrome - 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. The lipid A portion of endotoxin, which is the outer membrane lipopolysaccharide, initiates a chain of reactions, including the production of tumour-necrosis factor (TNF)¹⁴, interleukin-1 (IL-1) and complement activation, which contribute to the shock response seen in the patient. TNF is thought to be a principal mediator of toxic shock¹⁴.

Shock may also be associated with Gram positive organisms, particularly with fulminant pneumococcal, Lancefield Group A streptococcal and staphylococcal bacteraemia. Although Gram positive organisms lack lipopolysaccharide, other cell wall components such as peptidoglycan can produce similar effects³. The action of the toxins is not well understood, but it is proposed that the toxins act as super-antigens which cause massive T-lymphocyte proliferation and production of IL-1 and TNF¹⁴.

Antimicrobial agents are of little help in combating the acute effects of shock, and other supportive measures, such as mechanical ventilation and the maintenance of blood pressure, are essential.

Pseudobacteraemia occurs when blood culture isolates originate from outside the patient's bloodstream. Blood culture contamination may occur at any stage between the taking of the blood and processing in the laboratory, and can originate from a variety of sources. Outbreaks of pseudobacteraemia have been described. They usually involve contamination of fluids with environmental organisms, or incorrect sampling of blood¹⁵⁻¹⁸.

Bloodstream infections in patients who are immunocompetent

Community-acquired bacteraemia 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* osteomyelitis³. Other generalised bacteraemic illnesses include enteric fever (eg typhoid) and brucellosis.

Organisms most commonly isolated from adults with community acquired bacteraemia include^{2,3,13,19}:

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

Chronic meningococcaemia will occur occasionally in patients with inherited defects in humoral defence mechanisms such as complement deficiencies²⁰. Intermittent febrile episodes of 2-10 days duration are associated with a variety of skin lesions and migratory arthralgias and myalgias. Organisms may be isolated continuously or intermittently from the blood. Infection may last for months and may resolve spontaneously, but response to appropriate antibiotic therapy is dramatic.

Disseminated gonococcal infection (DGI)²¹ is a bacteraemic illness which develops in approximately 1-2% of patients with untreated gonorrhoea. Symptoms can include asymmetric polyarthritides, arthralgia, myalgia and a characteristic dermatitis, although the patient is often not clinically toxic. Asymptomatic mucosal infection is often present, so potentially infected sites (ie urethra, cervix, pharynx, rectum) should be investigated if DGI is suspected. Care must be taken in the interpretation of Gram negative diplococci in microscopy of positive blood cultures in clinical situations where gonococcal infection is possible. The Gram appearance of gonococci and meningococci will be the same therefore identification is important. This is because meningococci can produce similar clinical conditions (eg septic arthritis).

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Hospital-acquired bacteraemia - The increasing number of invasive procedures such as catheterisation, immunosuppressive and antibiotic therapy, and life support measures, have resulted in an overall increase in hospital-acquired bacteraemia. These procedures may introduce bacteria to the blood stream or may weaken the host defences. Organisms most frequently isolated from adults with hospital-acquired bloodstream infection will depend on the patient group and change with the duration of stay in hospital. Organisms include^{13,16,19}:

- 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^{3,22-30}.

Anaerobic bacteraemia still occurs despite the widespread use of prophylactic and therapeutic antibiotics with good activity against anaerobic organisms^{31,32}.

Although rare, necrobacillosis (Lemière's Syndrome) is a severe form of anaerobic septicaemia caused mainly by *Fusobacterium* species. The infection is most common in previously healthy young adults, and is characterised by a sore throat followed by suppurative endophlebitis of the jugular venous system, rigors and septicaemia^{32,33}. The condition may be rapidly fatal if untreated.

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 are similar to those seen in adults. Polymicrobial and anaerobic bacteraemia occur less frequently in children than in adults³⁴.

Occult bacteraemia³⁵ can occur in children with few or none of the symptoms normally associated with bloodstream infection. 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.

Lancefield Group B streptococci, *E. coli*, coagulase-negative staphylococci and *Candida* species remain the most common isolates from the blood in neonates.

Problems associated with the diagnosis of bacteraemia in children and neonates include specimen collection, small blood volume, number and timing of samples, and contamination. The concentration of organisms in the blood stream of children and neonates is generally higher than in adults although concentrations as low as 500 colony-forming units per litre (cfu/L) have been described^{34,36-38}.

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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 cultures³⁹.

Diagnosis of catheter-related bacteraemia is usually based on⁴⁰:

- 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
- Differential quantitative culture (≥ 10 -fold more organisms in blood drawn through the catheter than from a peripheral vein)⁴¹
- The differential time to positivity for through-line and peripheral blood cultures incubated on automated systems can also be used to diagnose catheter-related bacteraemia⁴²

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, acquired immune deficiency syndrome (AIDS) or sickle cell disease, and in patients who have had organ transplantation, immunosuppressive therapy or steroids⁴³. The risk of infection is greatest in patients with neutropenia.

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

Hyposplenic or asplenic patients are susceptible to fulminating septicaemia caused by a variety of organisms, particularly capsulate bacteria such as *S. pneumoniae*⁴⁴, *H. influenzae*, and *N. meningitidis*, but also less common organisms such as *Capnocytophaga* species³⁰.

The spectrum of organisms detected has changed in recent years. This probably 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^{30,45,46} and the number of Gram positive and opportunistic infections, particularly those caused by fungi and *Mycobacterium* species, has also increased. In addition to the organisms associated with bloodstream infection in the immunocompetent, isolates include⁴⁵:

- Non-fermentative Gram negative rods
- *Listeria monocytogenes*
- *Corynebacterium* species
- *Candida* species and other fungi
- *Mycobacterium* species
- Viruses

Other more unusual organisms include a variety of bacteria and fungi⁴⁷⁻⁵², many of which have very specific growth requirements. Viruses are a common cause of infection in organ transplant patients⁵³.

***Mycobacterium* species infection**

Mycobacterium species infection is becoming increasingly common as the incidence of patients with human immunodeficiency virus (HIV) and AIDS increases⁵⁴. For further details see BSOP 40 – Investigation of specimens for *Mycobacterium* species.

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Infective endocarditis^{53,55-58}

Infective endocarditis (IE) is the 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. A fibrin clot is deposited on the damaged endocardial surface and becomes colonised with organisms which have entered the bloodstream, so forming an infected vegetation. Viable bacteria may be present deep within the vegetation as well as on the surface making antimicrobial treatment difficult.

The disease used to be classified as either "acute" or "subacute", relating to the usual course of the untreated disease. Acute endocarditis was used to describe colonisation of normal heart valves by virulent bacteria, which led to rapid valve destruction, widespread metastatic foci, heart failure and rapid death. Subacute endocarditis described infection of abnormal valves by less virulent organisms, often being an insidious process, with metastatic foci uncommon. However, such classic presentations occur infrequently and this terminology is no longer used. Proposed in 1994, the Duke criteria are used as the diagnostic criteria⁵⁶⁻⁵⁸. 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. Most cases (60-80%) occur in middle aged to elderly patients with a cardiac lesion⁵⁹, the mitral valve being most commonly affected⁵⁵. Infective endocarditis can occur on anatomically and functionally normal valves as a sequel to certain bacteraemias. For example, up to 20% of patients who have *S. aureus* septicaemia develop endocarditis despite having normal valves.

Organisms most commonly isolated include⁵⁵:

- Oral streptococci
- Staphylococci (approximately 80% of these are *S. aureus*)
- Enterococci
- *Streptococcus bovis*

Fungal infection is rare, except in intravenous drug abusers and patients with severe underlying illnesses^{60,61}. 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*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*; see BSOP ID 12 – Identification of *Haemophilus* species and the HACEK group of organisms)⁵⁶⁻⁵⁸. *Bartonella* species are becoming increasingly important causes of endocarditis particularly in patients with AIDS¹².

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. Approximately 2% of patients with prosthetic valves develop endocarditis⁶², and PVE accounts for up to 20% of all cases of endocarditis⁵⁵. The prosthetic aortic valve is more prone to infection⁵⁵. Infection of the prosthetic valve may occur at any time after valve surgery, but becomes progressively less common as time passes and involves a different group of organisms.

"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 replacement³, reflecting contamination of the valve prosthetics arising 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.

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The most commonly isolated organisms are:

- Coagulase-negative staphylococci
- *S. aureus*
- Gram negative rods
- *Candida* and *Aspergillus* 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. Oral streptococci and staphylococci are the most common isolates, with a lesser incidence of infection caused by Gram - negative rods, *Candida* and *Aspergillus* species, enterococci and *Corynebacterium* specie.

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

Guidelines for managing unknown, unusual illnesses and deliberate release situations are available at <http://www.hpa.org.uk>

Bacillus anthracis

The organism will grow in all standard blood culture systems. All large, non-haemolytic, non-motile Gram positive rods present in blood cultures, CSF etc. must be treated with suspicion, as possibly being *B. anthracis*, with further initial investigation conducted under Containment Level 3 conditions. Strains of presumptive *B. anthracis* must be sent to an appropriate Reference Laboratory for further characterisation.

Refer to [BSOP ID 9 - Identification of *Bacillus* species](#)

***Brucella* species**

Brucella species appear as tiny catalase positive Gram negative coccobacilli. Growth is usually possible on rich media such as chocolate blood agar incubated at 37°C in 5% CO₂. Smooth, shiny colonies of 1mm or less in size are produced at 48 – 72 hours incubation. The oxidase and urease reactions are usually positive. These characteristics should prompt the laboratory to proceed with caution, and carry out any other work in Containment Level 3⁶³. Presumptive *Brucella* species must be sent to an appropriate reference laboratory for further characterisation.

Note: *Brucella* species may be mis-identified by semi-automated or kit-based identification systems as *Moraxella* or *Haemophilus* species.

***Francisella tularensis* (sensu lato)**

Francisella species are very dangerous to handle in the routine clinical laboratory. If the diagnosis is suspected on clinical or epidemiological grounds, it is recommended that attempts to isolate the bacterium are suspended, pending discussion with an appropriate Reference Laboratory.

Francisella species appear as small pleomorphic Gram - negative coccobacilli. There is little information regarding the ability of commercially-available blood culture systems to support the growth of this group of bacteria. Note that most strains of *F. tularensis* require cysteine for growth, and thus will not grow on most routine solid media. Positive blood cultures containing small non motile Gram - negative bacilli that fail to grow on conventional media might be subcultured (at Containment Level 3) to buffered charcoal yeast extract (*Legionella* isolation) media, chocolate blood agar, and gonococcus selective agars, all incubated 5% CO₂ enriched atmosphere, for up to 10 days. Colonies are small (1-2 mm in diameter), grey, smooth and shiny in appearance. *Francisella* species are slowly catalase positive. The oxidase test is negative.

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Burkholderia mallei

B. mallei is dangerous to handle in the clinical laboratory. If the diagnosis is suspected on clinical or epidemiological grounds, any further work must proceed at Containment Level 3. Putative isolates of *B. mallei* must be referred to an appropriate Reference Laboratory for confirmation.

There is little published information regarding *B. mallei* in the current biomedical literature. It is likely, however, that *B. mallei* will grow in commercially-available blood culture systems, and be recovered on standard laboratory media.

The oxidase reaction is given as variable⁶⁴ but the organism is always non-motile. *B. mallei* is listed in the databases of commercially-available kit-based identification systems, but the results must be interpreted with caution.

Burkholderia pseudomallei

The organism is dangerous to handle, but probably less so than *B. mallei*. However, once its presence is suspected, work should proceed at Containment Level 3. Putative isolates of *B. pseudomallei* must be referred to an appropriate Reference Laboratory for confirmation.

B. pseudomallei will grow in commercially available blood culture systems, and on standard laboratory media⁶⁴. Gram stained films of cultures and of clinical material reveal small, bipolar staining Gram negative rods. The oxidase reaction is positive, and the organism is motile. After a few days incubation, colonies may become rugose and wrinkled in appearance, resembling those of *Pseudomonas stutzeri*, but this is not a constant feature.

B. pseudomallei is listed in the databases of a number of commercially available kit-based identification systems, but results should be interpreted with caution.

Yersinia pestis

The organism is dangerous to handle in the clinical laboratory. Once its presence is suspected, work should proceed at Containment Level 3. All putative strains of *Y. pestis* must be referred to the appropriate Reference Laboratory for confirmation.

Y. pestis will grow in commercially available blood culture systems, and on standard laboratory media, including *Yersinia* selective (cefsulodin-irgasan-novobiocin) agar. Gram stained films of cultures and of clinical material reveal small, often bipolar-staining Gram negative rods as single organisms, or in short chains of bacilli. The oxidase reaction is negative, the catalase reaction is positive, and the organism is non-motile.

The optimal temperature for growth is 28 – 30°C but it will grow in temperatures up to 35°C. The organism is generally slow-growing. Maximum colonial size is generally reached at 2 – 3 days incubation on blood agar. Shiny, non-haemolytic colonies 2mm or so in diameter are formed, resembling “fried eggs”. The surface of these colonies is said to have a “hammered copper” appearance.

Y. pestis is listed in the databases of some commercially available kit-based identification systems, but results should be interpreted with caution.

Diagnosis of Bacteraemia and Fungaemia

The detection of bacteraemia and fungaemia requires a good blood culture system complemented by good laboratory practice and communication.

Blood culture systems

Should aim to achieve the following⁶⁵:

- 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

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- Minimisation of contamination
- Earliest possible detection of bacteria and fungi

Blood culture systems range from "in house" and commercial manual systems to fully automated commercial systems. These 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 advantages⁶⁶⁻⁷⁷. Fully automated continuous monitoring systems are simple to use in comparison with manual and semi-automated systems. In addition, positive cultures may be detected earlier. This is particularly true of organisms considered most pathogenic eg *S. aureus*, Gram negative rods and streptococci⁷⁸.

Diphasic systems have the advantage of simple closed sub-culture, achieved by tilting of the bottle, but colony recognition may be impaired by the glass^{79,80}.

As a result of the decline in bacteraemia caused by anaerobic organisms, there is debate over whether anaerobic culture should be included selectively or on specimens from all patients^{9,81}. The authors of a study proposed that the use of an anaerobic bottle should be based on the recovery of bacteria and yeasts in general and of the recovery of specific organisms as well as the system and media used⁸².

Most systems employ both aerobic and anaerobic bottles for adults, but provide a single aerobic bottle for use with children, in whom anaerobic bloodstream infection is rare and blood specimen volumes obtained are often small.

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^{3,9,38,83}.

Clinical:

- Method of collection
- Number and timing of samples
- Previous antimicrobial therapy

Technical:

- Volume of sample
- Media used
- Neutralisation of antimicrobial agents
- Incubation time and temperature
- Agitation of media
- Headspace atmosphere

Collection

Collection of blood should be performed from a suitable venepuncture site, following disinfection of the skin. Changing needles between venepuncture and inoculation of the bottles is not recommended as this carries a risk of needlestick injury. Needle changing does not reduce contamination rates according to some⁸⁴⁻⁸⁶, but slightly reduces contamination according to a meta-analysis⁸⁷. Arterial blood offers no advantage over venous blood for most micro-organisms, although it has been reported as being superior in detecting disseminated fungal disease⁸⁸.

Number and timing of samples

A second or third blood culture set not only increases yield but also allows recognition of contamination⁷. For the majority of patients, two blood culture sets are recommended³⁸. 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

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as soon as possible after a spike of fever⁸. However, some work has shown little difference in isolation rates between blood drawn at intervals and simultaneously⁸⁹. Certainly, the timing is less important for continuous bacteraemia, as seen in IE.

Volume of blood

The volume of blood cultured is the most critical factor in the detection of bloodstream infection. The number of organisms present in adult bacteraemia is frequently low, often $<1 \times 10^3$ colony forming units per litre (cfu/L)^{90,91}. There is a direct relationship between blood volume and yield, with approximately a 3% increase in yield per mL of blood cultured⁹¹. It is recommended that 20-30mL blood be cultured. Whereas most modern commercial systems allow 10mL blood to be added to each bottle, a few restrict volumes to 5mL per bottle. In infants and children the magnitude of bacteraemia is usually higher than that in adults, although low level bacteraemia ($<4 \times 10^3$ cfu/L) does occur^{36,92}. A volume of 1-2mL is recommended for neonates³⁴.

Media

Most systems employ different media for the isolation of aerobic and anaerobic organisms, and some media are specifically designed for organisms such as fungi and *Mycobacterium* species. A variety of blood culture media are commercially available and have been reviewed^{38,73,93}. They differ in the types and proportions of various supplements and anticoagulants, volumes of broth, headspace atmospheres and the presence of antimicrobial-neutralising agents. The aerobic bottle may or may not require transient venting to increase the oxygen content in the headspace for strictly aerobic organisms such as *P. aeruginosa* and *Candida albicans*⁹⁴⁻⁹⁷.

Blood:broth ratio

A blood to broth ratio of about 1:15 is required to remove the antibacterial effects of normal human blood, but this may be reduced to between 1:5 and 1:10 by the addition of 0.05% sodium polyanethol sulphate (SPS)⁹⁸. SPS has an inhibitory effect on *Neisseria* species, anaerobic cocci, *Streptobacillus moniliformis* and *Mycoplasma hominis*⁹⁹. The inhibitory effects of SPS may be reduced by the addition of gelatin to the broth^{100,101}. The medium in some commercially available bottles is supplemented with materials which improve microbial recovery by adsorbing antimicrobial substances and lysing the contained WBCs to release micro-organisms to the blood-broth mixture⁹³.

Neutralisation of antimicrobials

Neutralisation of antimicrobials may be achieved by several methods. The addition of beta-lactamase will help overcome the effect of beta-lactam antibiotics. Media containing resins or other adsorptive materials have been developed, and lysis-centrifugation techniques have been used, but there are conflicting reports concerning their efficacy^{77,102-106} and the clinical importance of the increased isolation rates attributed to them¹⁰⁷.

Incubation time and temperature

A temperature of 35-37°C for 5-7 days is recommended for routine blood cultures¹⁰⁸. Five days is usually sufficient incubation time for the recovery of most organisms if automated systems are used¹⁰⁹⁻¹¹¹. If conditions such as brucellosis and tularaemia are suspected the incubation period may need to be extended to ten days and include a terminal subculture¹¹²⁻¹¹⁶. 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 also need to be extended for some cases of suspected endocarditis, for patients on antimicrobial therapy, or when infection with fungi or unusual, fastidious or slow growing organisms is suspected¹¹⁷, but the increased yield is small¹¹⁸.

If due to an unavoidable delay bottles are incubated prior to loading on to the machine it should be aware that the machine may fail to detect certain Gram negative bacteria and streptococcal species. On these occasions the bottle should be checked visually for signs of growth prior to loading on to the machine. If growth is suspected then the bottles should be sub cultured.^{119,120}

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 CO₂

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and may require venting to increase the O₂ content. Depending on the system, the headspace of anaerobic bottles usually contains combinations of CO₂ and nitrogen.

Agitation of media

The effects of agitation are usually an increased yield^{121,122} and earlier recovery¹²¹. Agitation of anaerobic bottles does not increase yield, and agitation of mycobacterial blood cultures decreases yield¹²³. 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.

Subculture

If manual or semi-automated systems are used, subculture of both bottles in a set if only one bottle shows evidence of microbial growth will reveal both to be positive in about 50% of cases. However, this is probably unnecessary 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.

Blind or terminal subculture

is not recommended for routine blood cultures if automated systems are used,^{111,123} but may be indicated for manual systems¹²³. However, 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. They may fail to “flag” as positive, or detection of positivity may be delayed on automated systems. Similar effects have been reported for *P. aeruginosa* and *Candida* species¹¹⁶. Blind subculture (at appropriate containment level) of bottles from patients with a clinical likelihood of such organisms should be considered.

Inconsistent results

These include bottles with:

Positive appearance/flag positive with positive Gram film and negative subculture

Seen with *Abiotrophia* species (nutritionally variant streptococci)¹²⁴, *S. pneumoniae* which have undergone a degree of autolysis, and fastidious organisms which are unable to grow on routine solid culture media^{12,124-127}. Additional or supplemented media, prolonged incubation or alternative growth atmosphere should be considered, depending on the microscopy and clinical indications. Some media are reported to reduce the autolysis of *S. pneumoniae*¹²⁸. 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 film and 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's¹²⁹ may be required to demonstrate the physical presence and morphology. This may give guidance for the selection of appropriate media for subcultures.

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

Organisms may be present in the medium but show minimal or no positive growth criteria (see blind subcultures above). Investigation is usually based on a high degree of clinical suspicion. This may be the case with *Brucella*, *Francisella* or *Legionella* species.

Post-mortem blood cultures

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Blood cultures taken at post-mortem have been reported as diagnostically useful if taken under controlled conditions, particularly if the same organism is isolated as from ante-mortem blood cultures or from another site¹³⁰, but this has been disputed¹³¹. Results of post-mortem blood cultures should be interpreted with caution.

TECHNICAL INFORMATION/LIMITATIONS

Rapid detection and identification directly from blood culture broth

A variety of rapid methods have been evaluated and found to be useful under certain conditions, although none have been standardised¹³². Variable sensitivity and specificity have been reported, and may be medium dependent¹³³. An earlier result obtained by such means may or may not influence patient management, but could improve laboratory work-flow or reduce costs. Antigen testing of the blood culture broth may be useful in confirming the presence of *S. pneumoniae* that has undergone autolysis. However, it is important that results of direct testing of blood cultures using commercial or other products should be viewed with caution unless they have been evaluated and approved for such use.

UNDER CONSULTATION

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1 SAFETY CONSIDERATIONS¹³⁴⁻¹⁴⁴

1.1 SPECIMEN COLLECTION

Inspect the blood culture bottles for damage or defect before use.

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

Do not re-sheathe needles.

1.2 SPECIMEN TRANSPORT AND STORAGE

Transport specimens in a sealed plastic bag and in a rigid leak proof outer container.

If transport to a reference laboratory is required, the specimen must be packaged according to postal regulations.

1.3 SPECIMEN PROCESSING

All specimens should be processed in containment level 2 accommodation unless infection with a Hazard Group 3 organism eg *Mycobacterium tuberculosis*, *Brucella* species, *Francisella* species, *Y. pestis*, *B. mallei*, *B. pseudomallei* or subculture of blood culture bottles from suspected cases of typhoid/paratyphoid is suspected, in which case work should be performed in a microbiological safety cabinet under containment level 3 conditions.

Laboratory procedures that give rise to infectious aerosols must be performed in a microbiological safety cabinet. Although *N. meningitidis* is in Hazard Group 2, local policy may dictate that suspected isolates of *N. meningitidis* should always be handled in a microbiological safety cabinet. Sometimes the nature of the work may dictate the use of full containment level 3 conditions eg research work using *N. meningitidis* in order to comply with COSHH 2002 Schedule 1 5. (5e)

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 manufacturer's recommendations and local protocols.

Refer to current guidelines on the safe handling of all organisms documented in this SOP.

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

Compliance with postal and transport regulations is essential.

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME FOR SPECIMEN COLLECTION

Collect specimens before the start of antimicrobial therapy where possible.

Collect specimens as soon as possible after a spike of fever, except in endocarditis where timing is less important.

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

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.

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Disinfect the skin at the venepuncture site with ethanol or isopropyl alcohol and allow to dry.

Disinfect the septum of the blood culture bottle with ethanol, methanol or isopropyl alcohol and allow to dry (the use of iodine-based disinfectants is NOT recommended for some commercial systems as this is said to affect the integrity of the butyl rubber septum).

For diagnosis of bacteraemia

Withdraw blood from a peripheral vein and divide the sample equally among blood culture bottles. Samples should not be taken through an intravenous catheter or other access device^{8,145} unless no other access is available.

Children and neonates

Consider the use of a single paediatric bottle appropriate for small volumes of blood. If the paediatric bottle is unavailable, use a single aerobic bottle.

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

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

Adults

A set is defined as one or more bottles taken at any one time.

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

Preferably, a volume of 1-2 mL in neonates, 2-3 mL in infants and 3-5 mL in pre-teen children for each blood culture set³⁴ should be taken.

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

General

Take two sets during any 24 h period for each septic episode⁷. For neonates, take a single set aerobic bottle or special paediatric bottles.

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Blood cultures should be transported to the laboratory and incubated or loaded to the automated system as soon as possible.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Where there is a delay in transport to the laboratory and/or loading on the automated system, blood cultures should be incubated at 35-37°C as soon as possible after inoculation, pending processing, and must not be refrigerated. If an incubator is unavailable on the ward, storage at ambient temperature is preferable to refrigeration before transportation. Laboratory workers need to be aware that they need to check these bottles for signs of growth prior to loading them on to the machines. If signs of growth are visible they should be subcultured^{119,120}.

Automated systems

In order to minimise the risk of autolysis of certain organisms such as pneumococci, subculture bottles as soon as possible after a positive flag is detected.

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4 SPECIMEN PROCESSING

4.1 INCUBATION OF BOTTLES

Load all bottles to the automated system according to manufacturer's instructions.

Incubate the bottles for 5-7days.

Incubation times may need to be extended for specific clinical situations such as endocarditis, brucellosis and tularaemia.

4.2 APPEARANCE

Inspect the pre-incubated bottles visually for evidence of microbial growth.

4.3 MICROSCOPY

Positive bottles - 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).

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

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

Note 1: Some systems may not require mixing, but recommend subculture of the buffy coat layer

Disinfect the septum of the blood culture bottle by wiping with ethanol, methanol or isopropyl alcohol and allow to dry.

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 2: Refer to manufacturer's instructions with respect to preparing smears from charcoal-containing bottles

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

Note 3: To reduce the number of bottle entries and possible contamination of the broth, media may be inoculated at the same time as the film is prepared and/or a small aliquot may be transferred to a sterile tube/container for subsequent inoculation of additional media or antigen testing

Note 4: Gram negative organisms may be seen more easily if Sandiford's¹²⁹ or carbol fuchsin counterstain is used (see [BSOFTP 36 – Staining procedures](#))

If organisms are not seen on microscopy, investigate the growth curve (automated systems). If growth parameters indicate positive microbial growth, the preparation of further films with alternative stains may be useful.

If organisms are not seen on microscopy, subculture the bottle to agar plates (see 4.4.2) and return the bottle to the automated system, according to manufacturer's instructions, for further incubation and testing.

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

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Note : Small Gram negative rods may be *Brucella* or *Francisella* species; large Gram positive rods may be *B. anthracis* - refer to safety precautions

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

N/A

4.4.2 SPECIMEN PROCESSING

Standard

Positive bottles from all systems

Disinfect the septum of the blood culture bottle by wiping with ethanol, methanol or isopropyl alcohol 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. (QSOP 52 – Inoculation of Culture Media)

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 manual systems

Perform blind subculture for any aerobic bottle that appears negative after 24-48h¹⁰⁵.

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 film result, consider the possibility of a nutritionally dependent, slow growing or fastidious organism, such as:

- *Campylobacter* species
- *Brucella* species
- *Abiotrophia* species
- Cysteine-dependent organisms including *Francisella* species
- *Legionella* species
- *Helicobacter* species
- Slow-growing anaerobes

Subculture to appropriate media, or if uncertain as to possible aetiology, perform supplementary culture as indicated in Section 4.4.3.

Consider the possibility of autolysis (particularly common with *S. pneumoniae*). If there is a possibility of autolysis perform direct antigen testing for *S. pneumoniae* on the broth.

If blood appears lysed (in systems where blood is not normally lysed), subculture to fresh blood culture bottles in an attempt to recover the organism.

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4.4.3 MEDIA FOR SUBCULTURE, CONDITIONS AND ORGANISMS FOR ALL POSITIVE SPECIMENS AND BLIND SUBCULTURES:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Any clinical details	Blood agar	35-37	5-10% CO ₂	40-48h*	Daily	Any organism may be significant
	Fastidious anaerobe agar†	35-37	anaerobic	40-48h*	Daily	
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% CO ₂	40-48h*	Daily	<i>Haemophilus</i> species <i>N. meningitidis</i>
Gram - negative rods seen on microscopy	CLED/MacConkey agar or Chromogenic agar	35-37	air	16-24h	≥16h	Enterobacteriaceae
Microscopy suggestive of mixed or anaerobic infection	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	40-48h*	≥40h	Anaerobes
Systemic fungal infection	Sabouraud agar	28-30	air	5d	2d and at 5d	Fungi
Primary culture negative and positive growth curve‡	Blood agar	35-37	micro- aerobic	5d	≥3d and at 5d	<i>Campylobacter</i> species <i>Helicobacter</i> species
(subculture all bottles)	Blood agar with streak of <i>S.</i> <i>aureus</i> (NCTC 6571)	35-37	5-10% CO ₂	40-48h	≥40h	<i>Abiotrophia</i> species
	Fastidious anaerobe agar	35-37	5-10% CO ₂	5d	≥2d and at 5d	Cysteine-dependent organisms
	MACCONKEY agar	35-37	air	16-24h	≥16h	

Other organisms for consideration - Mycobacterium (BSOP 40) and Legionella species (BSOP 47) and Brucella species: also consider organisms that might be involved in deliberate release. (See Section 5.)

*incubation may be extended to 5 days if false-negative likely or as clinically indicated; in such cases plates should be read at ≥40h and left in the incubator/cabinet until day 5.

†an optochin disc may be added if streptococci seen on microscopy

‡other organisms may need to be considered

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4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL IN THE LABORATORY

All clinically significant isolates should be identified to species level with the exception of Coagulase Negative Staphylococci. In cases of endocarditis and deep seated abscesses 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 week¹⁴⁵. 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)

4.5.2 REFERRAL TO REFERENCE LABORATORIES

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms](#).

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

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Refer to [BSOP 45 – Susceptibility testing](#)

5 REPORTING PROCEDURE

5.1 MICROSCOPY

Gram stain

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

Other supplementary stains

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

5.1.1 MICROSCOPY REPORTING TIME

Positive results should be telephoned or sent electronically according to local protocols and therapy discussed where appropriate.

Written report should be issued 16-24h after bottles flag positive.

5.2 CULTURE

Following results should be reported:

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

5.2.1 CULTURE REPORTING TIME

Positive culture reports should be telephoned or sent electronically, stating, if appropriate, that a further report will be issued

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Negative results should be reported at or after 48 h

5.2.2 SUPPLEMENTARY INVESTIGATIONS

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

Streptococcus pneumoniae should be detected by antigen or PCR as appropriate.

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

5.4 SUSCEPTIBILITY TESTING

Report results of susceptibility testing as clinically indicated

6 REPORTING TO THE HPA¹⁴⁶ (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications:

"Laboratory reporting to the HPA: A guide for diagnostic Laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Suspicion of deliberate release of micro-organisms must be notified urgently to the Consultant for Communicable Disease Control at the Centre for Infections.

7 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the relevant NSMs available through the Department for Evaluations, Standards and Training web page (www.hpa-standardmethods.org.uk).

Other documents that may be of relevance to this NSM are:

[BSOP 26 – Investigation of fluids from normally sterile sites](#)

[BSOP 40 – Investigation of specimens for *Mycobacterium* species](#)

[BSOP 45 – Susceptibility testing](#)

[BSOP ID 9 - Identification of *Bacillus* species](#)

[BSOFTP 36 – Staining procedures](#)

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8 ACKNOWLEDGEMENTS AND CONTACTS

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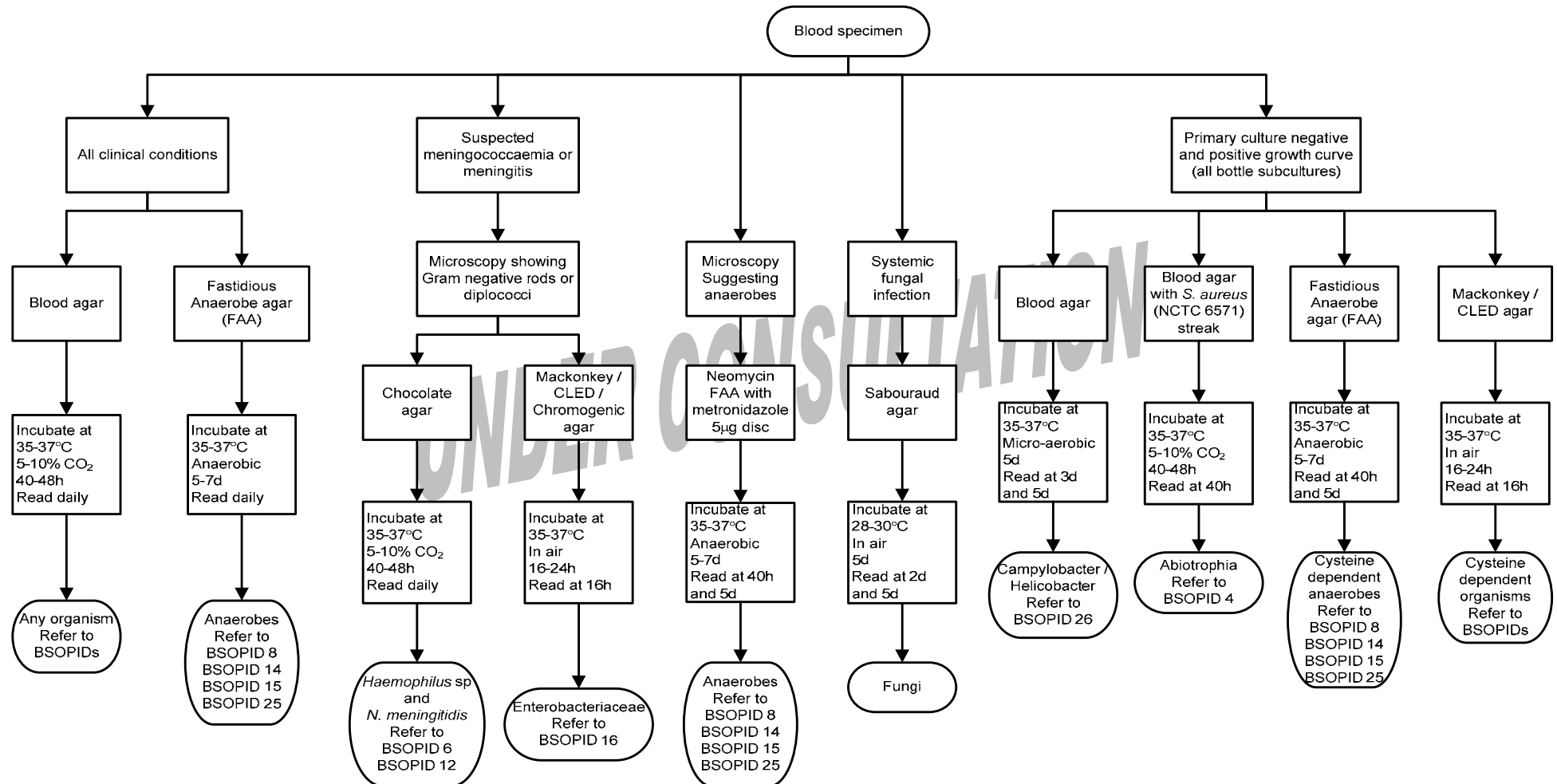
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APPENDIX



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