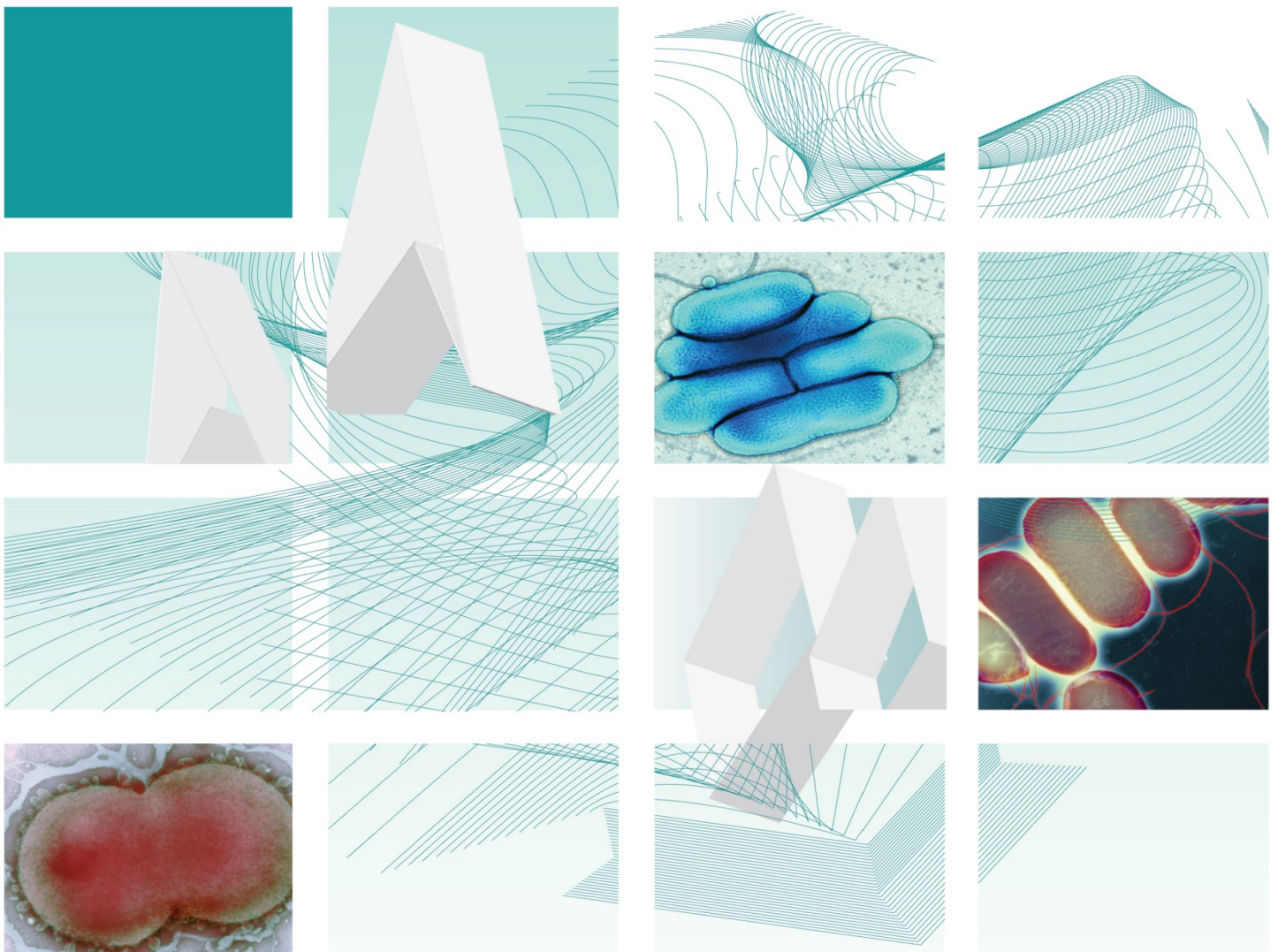




Protecting and improving the nation's health

# UK Standards for Microbiology Investigations

## Investigation of bone and soft tissue associated with osteomyelitis



NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

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## Acknowledgments

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UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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## 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](mailto: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.	4/14.12.15
Issue no. discarded.	1.3
Insert issue no.	2
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope.	Updated for clarity.
Introduction.	Re-organised and streamlined. Updated to include Waldvogel and Cierry-Mader classifications, spondylodiscitis and rapid techniques.
Technical information/limitations.	Updated to include limitations of UK SMIs.
Specimen transport and storage.	Section 3.1. IDSA Guidelines for transport included: Transport at room temperature, and should be processed immediately, and within a maximum of 2hr.
Specimen processing/procedure.	Section 4.3.1. Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised. Addition of information regarding molecular testing.
Culture and investigation.	Culture media, conditions and organisms updated. Direct FAA plate removed. Addition of molecular testing.
Reporting.	Reporting text updated. Addition of molecular testing.
Appendix.	Flowchart updated to reflect culture media table.



## UK SMI<sup>#</sup>: scope and purpose

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### Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard 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 laboratory processes which underpin quality, for example assay validation.

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 surveillance, research and development activities.

### Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

### Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

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<sup>#</sup> 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.

laboratories in the UK are expected to work. SMIs are NICE accredited 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. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. 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.

### **Patient and public involvement**

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

### **Information governance and equality**

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

### **Legal statement**

While every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

### **Suggested citation for this document**

Public Health England. (2015). Investigation of bone and soft tissue associated with osteomyelitis. UK Standards for Microbiology Investigations. B 42 Issue 2. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

## Scope of document

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### Type of specimen

Intra-operative samples of bone, bone biopsies, soft tissue, aspirates

This SMI describes the processing and microbiological investigation of bone and soft tissue associated with osteomyelitis and includes information regarding molecular techniques.

For biopsies and aspirates sent for the investigation of prosthetic joint infections refer to [B 44 – Investigation of orthopaedic implant associated infections](#).

This SMI should be used in conjunction with other SMIs.

## Introduction

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Osteomyelitis is a progressive infection which results in inflammation of the bone and causes bone destruction, necrosis and deformation<sup>1</sup>. In children the growing ends of long bones are the most common site of infection whereas in adults it is the spine<sup>2,3</sup>. Risk factors for adult (haematogenous) osteomyelitis include sickle cell disease, immune deficiencies and intravenous drug use<sup>4</sup>. Organisms most often isolated from bone and soft tissue samples include<sup>5</sup>:

- *Staphylococcus aureus*
- coagulase negative staphylococci
- *Enterococcus* species

Gram negative bacteria and fungi may also be isolated. Gram negative bacilli when isolated are of major clinical importance due to their antimicrobial resistance patterns<sup>6</sup>.

### Classification

Two classification systems of osteomyelitis are currently in use; the Waldvogel classification and the Cierny-Mader classification<sup>7,8</sup>.

The Waldvogel classification is based on the pathogenesis of disease. Categories are defined by the duration of illness (acute/chronic), the source of infection (eg contiguous focus originating from local infected tissue) and vascular insufficiency (eg diabetic foot infection)<sup>7,9</sup>.

There are several limitations to the Waldvogel classification; it does not include infection caused by direct inoculation into the bone, for example caused by trauma, and due to the fact that it is based on pathogenesis of disease, it does not lend itself to use in clinical practice<sup>9</sup>.

The Cierny-Mader classification is a clinical 'staged' classification. Initially cases are categorised into one of four stages of osteomyelitis; stage 1 (medullary), stage 2 (superficial), stage 3 (localised) or stage 4 (diffuse)<sup>9,10</sup>. The patient is then categorised as either A, B or C. Patients in category A do not have systemic or local compromising factors, those in category B are affected by systemic and/or local compromising factors and those patients in category C are severely compromised, and treatment is considered worse than the disease<sup>9</sup>.

There are also several limitations to the Cierny-Mader method of classification. Placing patients into category C is subjective and categorisation may differ between clinicians. Also, the classification does not take the duration of the disease into consideration<sup>9</sup>.

For the purpose of this SMI the structure of the introduction has been arranged based on the Waldvogel classification because of its etiological basis.

## Acute contiguous-focus osteomyelitis

In contiguous-focus osteomyelitis, the organisms may be inoculated at the time of trauma or during intra-operative or peri-operative procedures. Alternatively they may extend from an adjacent soft tissue focus of infection. Common predisposing factors include surgical reduction and fixation of fractures, prosthetic devices, open fractures and chronic soft tissue infections (see [B 14 – Investigation of abscesses and deep seated wound infections](#)). In general the microbiology of contiguous osteomyelitis is more complex than that of haematogenous osteomyelitis and is commonly polymicrobial.

### Contiguous-focus osteomyelitis without vascular insufficiency

Puncture wounds of the foot through footwear such as training shoes are particularly associated with osteomyelitis due to *Pseudomonas aeruginosa*<sup>11-13</sup>. Osteomyelitis following human bites and tooth socket infections affecting the mandible are often caused by strict anaerobes for example *Actinomyces* species; in children anaerobic bone and joint infections are rare<sup>14-16</sup>.

### Contiguous-focus osteomyelitis with vascular insufficiency

Most patients with contiguous-focus osteomyelitis associated with vascular insufficiency have diabetes mellitus. The bones and joints of the feet are most often affected<sup>9</sup>.

Diabetic foot infections are responsible for many hospital admissions and a significant number can end up with limb amputation and consequent disability<sup>17-19</sup>. Neuropathy and vasculopathy (impaired blood supply) are complications of diabetes. The former means that protective sensation is lost, allowing skin injury to occur without it being perceived. In addition it can ultimately lead to fragmentation, destruction and dislocations of the bones of the foot (Charcot neuro-osteoarthropathy). Foot deformity in diabetics due to motor neuropathy is also a further strong risk factor for developing ulcers and infection. The basic principles in the treatment of diabetic foot infection are education and prevention with good glucose control, accommodative footwear, regular inspection and general compliance.

Once infection has occurred, abscesses may need to be drained, diagnostic biopsies may be required to guide antibiotics and diseased bone may need to be resected. Acute infections in patients who have not recently received antimicrobials are often monomicrobial (almost always with aerobic Gram positive cocci such as *S. aureus* and  $\beta$ -haemolytic streptococci), whereas chronic infections are often polymicrobial. Cultures of specimens obtained from patients with such mixed infections generally yield 3–5 isolates, including Gram positive and Gram negative aerobes and anaerobes. These may include enterococci, various Enterobacteriaceae, obligate anaerobes, *Pseudomonas aeruginosa* and other non-fermentative Gram negative rods. Hospitalisation, surgical procedures, and, especially, prolonged or broad spectrum antibiotic therapy may predispose patients to colonisation and/or infection



with antibiotic resistant organisms (eg meticillin resistant *Staphylococcus aureus* (MRSA) or vancomycin resistant enterococci (VRE)). The impaired host defences around necrotic soft tissue or bone may allow low-virulence colonizers, such as coagulase negative staphylococci and *Corynebacterium* species (“diphtheroids”), to assume a pathogenic role.

In the immunocompromised or diabetic host, *Nocardia* species should also be considered as a rare cause of osteomyelitis<sup>20</sup>.

### Acute haematogenous osteomyelitis<sup>1,2,4,21</sup>

Haematogenous osteomyelitis has been classically described in childhood, but can occur in any age group especially when there are risk factors such as a recent intravascular device, haemodialysis, intravenous drug usage or recurrent infections elsewhere (such as urinary tract infections)<sup>4</sup>. In adults the vertebrae are most often affected, however the long bones, pelvis or clavicle may also be affected<sup>22</sup>.

In classical haematogenous osteomyelitis of childhood, the growing ends (metaphyses) of long bones are involved. The commonest organism is *S. aureus*; however  $\beta$ -haemolytic streptococci and HACEK organisms such as *Kingella* species are also important causes<sup>21</sup>. Organisms in the bloodstream gain access to bone by way of the nutrient artery. They pass through branches of this vessel to the small blind ended terminal vessels usually near the epiphyseal plate (growing end of the bone). This area is thought to have sluggish circulation, and bacteria can lodge here, starting the process of infection. Following this there is extension to other areas and the host inflammatory response is mobilised. Pus is created and expands under pressure thereby creating further impedance of the local circulation and death of bone.

In certain areas such as the hip, where the epiphyseal plate is situated within the joint capsule, early joint involvement by infection is common. Pus under pressure may strip the periosteum (outer lining of bone). New immature bone is formed as a response to periosteal stripping, and, in severe cases, the entire shaft may be encased in a sheath of new bone referred to as an involucrum. Where a major portion of the shaft has been deprived of blood supply, a resulting sequestrum (dead bone) lies within the involucrum. Openings in the bone may permit escape of pus from bone causing abscesses, systemic sepsis and in some cases death.

The bacterial species in haematogenous osteomyelitis are usually dependent on the age of the patient. In neonates, Group B streptococci, *S. aureus* and *Escherichia coli* cause infection<sup>1</sup>. Multiple sites of infection are common in neonates<sup>23</sup>. Between the ages of one and sixteen, *S. aureus*, and *Haemophilus influenzae* type b predominate (although the latter is rare after the age of five years and increasingly rare in children under five because of a successful vaccination campaign). *Streptococcus pneumoniae* is occasionally involved. In adult life, *S. aureus* is the commonest organism and, in the elderly, infection with aerobic Gram negative rods may occur. *Candida* species may be found when intravenous devices are in use<sup>24</sup>. In acute haematogenous osteomyelitis a single pathogenic organism is usually isolated but in many cases of chronic osteomyelitis, particularly when associated with wounds and ulcers the disease can be polymicrobial.

*Salmonella* species rarely cause osteomyelitis in patients who are immunocompetent; typically infections with *Salmonella* species (usually non-typhi serotypes) are associated with sickle cell anaemia (see below), other haemoglobinopathies or patients who are immunocompromised<sup>25</sup>. *Salmonella* osteomyelitis normally affects

the diaphysis of long bones (usually the femur or humerus) and the vertebrae<sup>25</sup>. Infection by indirect contamination from an animal host has been reported<sup>26,27</sup>.

### **Spondylodiscitis<sup>3</sup>**

The term spondylodiscitis refers to vertical osteomyelitis, discitis and spondylitis. These are manifestations of haematogenous osteomyelitis, which may result from the same pathological process and may occur at the same time<sup>3,28</sup>. Spondylodiscitis is responsible for 3-5% of all osteomyelitis cases, and it is the main cause of osteomyelitis in patients aged over 50 years<sup>3</sup>.

In adults, organisms may enter the discs via the arteries causing inflammation; infection may then extend to the vertebral column<sup>28,29</sup>. Vertebral osteomyelitis may also result from trauma or complications during surgery. Risk factors include older age, a recent intravascular device, haemodialysis, diabetes and intravenous drug usage (a risk factor for *Pseudomonas* infection), infection and immunosuppression<sup>30</sup>. Lumbar spine infections may originate from urinary tract infections, possibly by translocation of bacteria via a venous plexus (Batson's plexus) that links the bladder with the spine. Following the initial infection, pus may break out of the cortex anteriorly to form a paravertebral abscess or posteriorly to form an epidural abscess. In addition weakening of the bone may cause vertebral collapse. Organisms causing vertebral infections include *S. aureus*, streptococci and aerobic Gram negative rods (associated with urinary tract infections)<sup>1</sup>.

In patients with risk factors, tuberculosis should always be considered; microbiological diagnosis (with or without histology) is required for a definitive diagnosis<sup>31,32</sup>. Although infection most often occurs in the spine, extrapulmonary tuberculosis can occur in any bone or joint. Diagnosis is mainly by biopsy for histology and microbiology. Cultures are often positive and are crucial for determining the presence of resistance to anti-tuberculosis agents. However the decision to treat is often made on clinical and histopathological grounds in the first instance<sup>33,34</sup>. Other mycobacteria, such as *Mycobacterium marinum*, *Mycobacterium avium-intracellulare*, *Mycobacterium fortuitum* and *Mycobacterium gordonae* have also been associated with bone infections particularly in patients who are immunocompromised.

In endemic areas *Brucella* species are a common cause of vertebral infection, therefore a travel history should always be sought. Other fastidious Gram negative rods eg the HACEK group (*Haemophilus* species, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species (see [ID 12 – Identification of Haemophilus species and the HACEK group of organisms](#)) may be occasional causes of vertebral osteomyelitis<sup>35</sup>.

### **Sickle cell disease<sup>1,9</sup>**

Adult haematogenous osteomyelitis in adults is often associated with sickle cell disease. Symptoms may mimic those of marrow crisis; culture results should therefore be used for confirmation of clinical diagnosis. Organisms often isolated include *Salmonella* species, *S. aureus* and streptococci.

### **Haemodialysis patients<sup>9</sup>**

As a result of the use of intravascular access devices in these patients, haematogenous infections can occur usually due to *S. aureus* or coagulase negative staphylococci. Gram negative infections are more common in haemodialysis patients than in the general population.

### **Intravenous drug users<sup>9,36</sup>**

Septic arthritis and osteomyelitis of the long bones or vertebral discs are associated with haematogenous infection in intravenous drug users. Organisms often isolated include *S. aureus*, *P. aeruginosa* and *Candida* species.

### **Chronic osteomyelitis<sup>1,9,22</sup>**

Patients typically present with chronic pain and drainage, and may have a history of previous osteomyelitis at the same site. Treatment of infection may be challenging as surrounding tissue and bone will be of poor quality; antibiotic treatment alone is rarely sufficient to arrest infection. Risk factors include open fractures, bacteraemia and ischemic ulcers associated with diabetes, sickle cell disease and malnutrition. Organisms often associated with chronic osteomyelitis include *S. aureus*, Gram negative bacilli and anaerobic bacteria.

### **Device related osteomyelitis**

In chronic device related infections, organisms may be present in a biofilm that is associated with the device or diseased/necrotic bone. Refer to [B 44 – Investigation of orthopaedic implant associated infections](#).

### **Haematogenous**

Acute haematogenous osteomyelitis can lead to chronic osteomyelitis characterised by dead areas of bone and sinus tract<sup>37,38</sup>. This condition can fail to respond to treatment and persist for long periods<sup>10</sup>. Infections may recur many years after the first episode<sup>39</sup>.

### **Brodie's abscess<sup>40</sup>**

Brodie's abscess is an uncommon condition and is a chronic localised abscess of bone, most often in the distal part of the tibia. It is usually due to *S. aureus* and generally occurs in patients under 25 years of age. Surgery (surgical debridement) and culture-specific antibiotic therapy are usually effective in arresting infection.

### **Fungal osteomyelitis**

Fungal osteomyelitis is rare; however, some fungi endemic to certain areas can be associated with osteomyelitis. This includes *Cryptococcus*, *Blastomyces* and *Sporothrix* species. In patients who are immunocompromised or those with multiple previous surgical procedures at that site, more common fungi such as *Candida* or *Aspergillus* species can also cause osteomyelitis<sup>24,41</sup>. A mycetoma is a chronic granulomatous infection of the skin, subcutaneous tissues and in its advanced stages, bone. It is most prevalent in tropical and sub-tropical regions of Africa, Asia and Central America. Infection usually follows traumatic inoculation of organisms into subcutaneous tissue from soil or vegetable sources<sup>42</sup>. Various genera have been implicated including *Madurella*, *Acremonium*, *Pseudoallescheria* and *Actinomyces* species. Mycetoma may also be caused by bacteria<sup>43</sup>. It is characterised by subcutaneous granulomata containing grains and can lead to infection of the bone. Black grains are indicative of fungal infection and the condition known as eumycetoma. Actinomycetoma is caused by bacteria, and grains are white, yellow or red<sup>44</sup>. Infection normally occurs through a puncture wound (normally of the foot); however infection can occur in the legs, hands or arms<sup>43</sup>. Deep surgical biopsy is required to obtain viable samples for microbiological culture<sup>44</sup>.

## Diagnosis<sup>9,19,21,22</sup>

The diagnosis of osteomyelitis usually requires a combination of a full clinical assessment, plain X-rays and further imaging (eg MRI scan, CT scan, ultra-sound), blood cultures (particularly in acute cases), bone and/or soft tissue biopsies and/or surgical sampling. For specific indications eg risk of Brucella infection, other tests such as serology may be required. When tuberculosis is suspected, a full clinical 'work up' including a chest X-ray is indicated.

## Sample types

### Radiologically obtained percutaneous bone biopsies

These may be taken in the radiology department where they can be guided by imaging such as ultrasound, fluoroscopy or CT. Usually a sample should also be sent to histology to confirm infection, provide pointers to unusual infections and/or exclude malignancy. It is not commonly possible to send more than one sample to microbiology, but when this is done, each should be processed separately. It is important that detailed clinical information is provided to ensure cultures are set up for appropriate organisms. This includes details such as the presence of a prosthetic device (where any organism eg a coagulase negative staphylococcus, may be the pathogen and also where prolonged cultures are required). It also includes any clinical suspicion or risk factors for tuberculosis, brucella, nocardia, atypical mycobacteria or fungi.

### Intra-operative bone biopsies

These are taken in theatre either as primarily a diagnostic procedure, or as the first part of a larger debridement/resection procedure. Multiple (4-5) samples should be taken from separate site using separate sterile instruments for microbiological culture. Similar samples from similar sites should also be taken for histopathological examination. A risk-benefit assessment of antibiotic timing is required. Where infection is likely and/or a microbiological diagnosis is likely to significantly affect clinical outcome, prophylactic antibiotics can be withheld until immediately after sampling. The effect of a single dose of antibiotic on the sensitivity of microbiological culture is unknown. In addition to bone samples, deep soft tissue samples are usually taken at the same time. Sinus samples should be discouraged as colonising organisms cannot be differentiated from infecting organisms.

### Samples from around devices<sup>45</sup>

Samples of bone and soft tissues may be taken from around a prosthetic device, eg a fracture fixation plate or nail. Samples associated with such devices should be processed with the same principles as those associated with prosthetic joint samples ([B 44 – Investigation of orthopaedic implant associated infection](#)).

## Enrichment culture

Blood culture systems, where bottles are incubated for up to five days, have shown equivalent sensitivity to conventional enrichment broth for the culture of orthopaedic implant associated specimens (refer to [B 44 – Investigation of orthopaedic implant associated infection](#))<sup>46,47</sup>. Similar studies have not yet been published regarding their use for bone and soft tissue specimens associated with osteomyelitis.

## Management<sup>9,19,21,22</sup>

In acute presentations, surgery may be required to drain pus. In chronic osteomyelitis, areas of dead bone may need to be resected. Both need to be accompanied by specific antibiotic therapy depending on culture results. This is most often carried out intravenously, initially. In some cases, where the disease is too extensive to fully resect, the patient is too unfit for surgery, or a device is retained, long term oral antibiotics may be required. Organisms need to be tested against a wide variety of antibiotic options as patients commonly are intolerant of one or more antibiotics.

## Rapid techniques<sup>48-50</sup>

### Molecular methods<sup>50-52</sup>

Nucleic acid amplification techniques - NAAT (eg PCR) for the identification of bacteria, fungi, parasites and viruses from clinical samples have been shown to be highly specific and sensitive<sup>50,51,53</sup>. PCR targets conserved genes within the genome, and enables the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short timeframe particularly if multiplex real-time PCR is used.

PCR has been shown to be more sensitive than conventional culture for the isolation of some fastidious organisms for example *Kingella kingae*, and PCR – hybridization after sonication has been shown to improve diagnosis of implant related infections<sup>54,55</sup>. There are however some issues with NAATs analysis. A lowered sensitivity may be observed due to the small volume of samples processed, in some cases there may be interference with human DNA originating from the tissue samples, and antibiotic susceptibility information is not available<sup>56,57</sup>.

### MALDI-TOF mass spectrometry<sup>48,49</sup>

Recent developments in identification of bacteria and yeast include the use of 16s ribosomal protein profiles obtained by matrix assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectrometry. 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, rapid and effective identification system for bacterial and yeast isolates.

## Technical information/limitations

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### Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

### Specimen containers<sup>58,59</sup>

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”

## 1 Safety considerations<sup>58-74</sup>

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### 1.1 Specimen collection, transport and storage<sup>58-63</sup>

Use aseptic technique.

Care should be taken to avoid accidental injury when using “sharps”.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

### 1.2 Specimen processing<sup>58-74</sup>

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>66</sup>.

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 Specimen collection

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### 2.1 Type of specimens

Bone, bone biopsies, soft tissue, aspirates

### 2.2 Optimal time and method of collection<sup>75</sup>

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible<sup>75</sup>.

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium<sup>76-80</sup>.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Direct collection in theatres can be placed into a CE marked leak proof container with Ringer's or saline solution and Ballotini beads (as an option) which is placed into sealed plastic bags. However, microbiology and histology specimen pots can be confused leading to difficulties in processing samples.

### 2.3 Adequate quantity and appropriate number of specimens<sup>75</sup>

In surgery for chronic osteomyelitis collection of multiple (4-5) intra-operative samples with separate instruments (usually sterile forceps and scalpel) is important. Duplicate samples must be taken for histology. Swabs are not recommended.

Minimum specimen size will depend on the number of investigations requested.

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

## 3 Specimen transport and storage<sup>58,59</sup>

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### 3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible<sup>75</sup>. To enable timely clinical management, samples should be processed urgently.

The Infectious Diseases Society of America (IDSA) guidelines recommend that specimens should be transported at room temperature, and should be processed immediately, and within a maximum of 2hr<sup>75</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature.

If possible stop all antibiotics at least 2 weeks prior to sampling and consider not giving routine surgical prophylaxis until after sampling<sup>81</sup>.

The volume of the specimen influences the transport time that is acceptable. Larger pieces of bone may maintain the viability of anaerobes for longer<sup>82</sup>. Samples should not however exceed the size of the CE marked leak proof containers available.

## 4 Specimen processing/procedure<sup>58,59</sup>

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### 4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for *Mycobacterium* species ([B 40 - Investigation of specimens for \*Mycobacterium\* species](#)) or fungi depending on clinical details.

### 4.2 Appearance

N/A

### 4.3 Sample preparation

To enable timely clinical management, samples should be processed urgently. Non-repeatable samples should be prioritised.

For safety considerations refer to Section 1.2.

#### 4.3.1 Pre-treatment

Examine the specimen for the presence of any soft tissue. Remove soft tissue using a sterile scalpel or scissors and homogenise using, as appropriate, a sterile grinder (Griffith tube or unbreakable alternative), a sterile scalpel or (preferably) sterile scissors and Petri dish. The addition of a small volume (approximately 0.5mL) of sterile filtered water, saline or nutrient Ringer's will aid the homogenisation process.

Homogenisation must be performed in a racked shaker for 15 minutes in a Class 1 exhaust protective cabinet.

**Note:** Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised<sup>83</sup>.

#### Optional

N/A

## Supplementary

Fungi and *Mycobacterium* species ([B 40 - Investigation of specimens for \*Mycobacterium\* species](#)).

### 4.3.2 Specimen processing

#### Standard

##### **Bone (percutaneous biopsy or intra-operative sample) or soft tissue associated with osteomyelitis**

The objective should be to reduce manipulation to a minimum (for instance the number of times any container is opened), thereby minimising the risk of exposing the operative sample to potential contamination. For this reason centrifugation of the sample for concentration should not be performed, instead divide the whole sample in appropriate amounts for tests.

In units with high workloads of this specimen type, the provision to the operating theatre of CE marked leak proof containers in a sealed plastic bag with approximately 10 Ballotini beads and 5mL broth could be considered. In such circumstances, homogenisation can be carried out in the original container. It is not uncommon, however, for microbiology and histology specimen pots to be confused leading to difficulties in processing samples.

Alternatively, samples may be sent to the laboratory in a plain CE marked leak proof container in a sealed plastic bag. These samples require transfer, homogenisation and then further transfer to culture media, including liquid media. If this methodology is followed, particular care is necessary with asepsis when transferring, homogenising or processing the sample. Clean air provision is desirable. Homogenisation with Ballotini beads can be performed by adding the sample to a universal with approximately 10 Ballotini beads and 5mL of sterile saline (or Ringers solution) then shaking at 250 rpm for 10 minutes in a covered rack on an orbital shaker, or alternatively vortexing for 15 seconds (40 Hz). If molecular analysis is to be carried out then sterile molecular grade water and new universal containers should be used. In the case of molecular work the volume should not exceed 2mL.

Inoculate each agar plate and a slide for Gram staining with a drop of the suspension using a sterile pipette (see [Q 5 - Inoculation of culture media for bacteriology](#)).

For the isolation of individual colonies, spread inoculum using a sterile loop. Inoculate broth with the remainder of the suspension including any tissue fragments.

#### Optional

Specimens collected into appropriate CE marked leak proof containers should be used for microscopy and may be used for molecular techniques. Specimens for molecular testing should be processed according to manufacturer's instructions.

## Supplementary

Fungi and *Mycobacterium* species ([B 40 - Investigation of specimens for \*Mycobacterium\* species](#)).

## 4.4 Microscopy

### 4.4.1 Supplementary

**Gram stain** (see [TP 39 - Staining procedures](#))

Gram stains should be carried out on all pus samples and may be carried out on other sample types where clinically indicated. If sufficient specimen is received prepare as recommended in Section 4.5. Using a sterile pipette place one drop of specimen on to a clean microscope slide.

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

**Note:** Gram stain on tissue can be difficult to interpret and yield can be low.

## 4.5 Culture and investigation

Inoculate each agar plate using a sterile pipette ([Q 5 - Inoculation of culture media for bacteriology](#)).

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



## 4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
†Osteomyelitis Brodie's abscess, Diabetic foot osteomyelitis, Discitis +For debridement of fracture fixation device refer to <a href="#">B 44 – Investigation of prosthetic joint infection</a> .	Bone, Bone biopsy Soft tissue Aspirate	Blood agar and Chocolate agar	35 - 37	5 - 10% CO <sub>2</sub>	40 - 48hr	Daily	Staphylococci Streptococci Enterobacteriaceae Pseudomonads HACEK group <i>Nocardia</i> species*
		Fastidious anaerobic broth, cooked meat broth or equivalent Subculture onto plates if evidence of growth, or at day 5 as below:	35 - 37	Air	5 d	N/A	Staphylococci Streptococci Enterobacteriaceae Pseudomonads Anaerobes
Subculture plates	Bone, Bone biopsy Soft tissue Aspirate	FAA	35 - 37	Anaerobic	40 - 48hr	≥40hr	Anaerobes
		Chocolate agar	35 - 37	5 - 10% CO <sub>2</sub>	40 - 48hr	Daily	Any

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Deep seated fungal infection <sup>#</sup>	Bone, Bone biopsy Soft tissue Aspirate	Sabouraud agar (slopes)	28 - 30	Air	14 d	Daily‡	Yeast Mould

Optional molecular techniques

Clinical details/ conditions	Specimen	Molecular technique	Instructions	Target organism(s)
All clinical conditions	Bone marrow	NAAT	Follow manufacturer's instructions	Any organism

Always consider other organisms such as *Mycobacterium* species ([B 40 - Investigation of specimens for \*Mycobacterium\* species](#)), fungi and actinomycetes. Routine processing for mycobacteria should be considered for all non-post operative spinal infections.

\* If infection with *Nocardia* species is suspected, samples may require incubation for a further 3 days.

\*\* Subcultures should be examined periodically (ideally daily) and subcultured if there is evidence suggestive of growth. Terminal subcultures should be performed at 5 days.

+Most surgical cases with intra-operative biopsies eg fracture fixation devices or chronic osteomyelitis requires multiple samples. If an indistinguishable organism is isolated in two or more samples then it is likely to be clinically significant.

‡ Extended incubation may be required (for up to 8 weeks) for certain species of fungi such as *Cryptococcus* species or *Histoplasma* species<sup>84,85</sup>.

# When investigating mycetoma, deep surgical biopsy is required to obtain viable samples for microbiological culture. Samples should be submitted in normal saline<sup>44</sup>. (Refer to [B 17 – Investigation of tissues and biopsies](#)).

## 4.6 Identification

Refer to individual SMIs for organism identification.

### 4.6.1 Minimum level of identification in the laboratory

Actinomycetes	genus level <a href="#">ID 15 – Identification of anaerobic <i>Actinomyces</i> species</a>
Anaerobes	genus level <a href="#">ID 14 - Identification of anaerobic cocci</a> <a href="#">ID 8 - Identification of <i>Clostridium</i> species</a> <a href="#">ID 25 - Identification of anaerobic gram negative rods</a> <a href="#">ID 15 – Identification of anaerobic <i>Actinomyces</i> species</a>
<a href="#">β-haemolytic streptococci</a>	Lancefield group level
<a href="#">Other streptococci</a>	species level
<a href="#">Enterococci</a>	species level
<a href="#">Enterobacteriaceae</a>	species level
Yeast and Moulds	species level
<a href="#">Haemophilus</a>	species level
<a href="#">Pseudomonads</a>	species level
<a href="#">S. aureus</a>	species level
<a href="#">Staphylococci</a> (not <i>S. aureus</i> )	genus level
<a href="#">Mycobacterium species</a>	<a href="#">B 40 - Investigation of specimens for <i>Mycobacterium</i> species</a>

Organisms may be further identified if this is clinically or epidemiologically indicated.

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

## 4.7 Antimicrobial susceptibility testing

Refer to [British Society for Antimicrobial Chemotherapy \(BSAC\)](#), and/or [EUCAST](#) guidelines, or CSLI guidelines where applicable.

It is important to include a wide range of antibiotics particularly for those patients who may require prolonged oral treatment with biofilm active drugs (see Introduction). These antibiotics are not usually included in the common first line antimicrobials tested in most laboratories. For Gram positive organisms these may include a teicoplanin MIC plus antibiotics such as rifampicin, tetracyclines, quinolones, co-trimoxazole, fusidic acid, linezolid or quinupristin/dalfopristin.

## 4.8 Referral for outbreak investigations

N/A

## 4.9 Referral to reference laboratories

For information on the tests offered, turnaround 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, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

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

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.publichealth.hscni.net/directorate-public-health/health-protection>

## 5 Reporting procedure

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### 5.1 Microscopy

#### Standard

#### Gram stain

Report on WBCs and organisms detected.

#### 5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

### 5.2 Culture

Following results should be reported:

- clinically significant organisms isolated
- other growth
- absence of growth

#### 5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Also, report results of supplementary investigations.

Supplementary investigations: [B 40 - Investigation of specimens for \*Mycobacterium\* species](#).

### 5.3 Molecular

Refer to manufacturer's instructions.

### 5.4 Antimicrobial susceptibility testing

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

## 6 Notification to PHE<sup>86,87</sup>, or equivalent in the devolved administrations<sup>88-91</sup>

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The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (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.

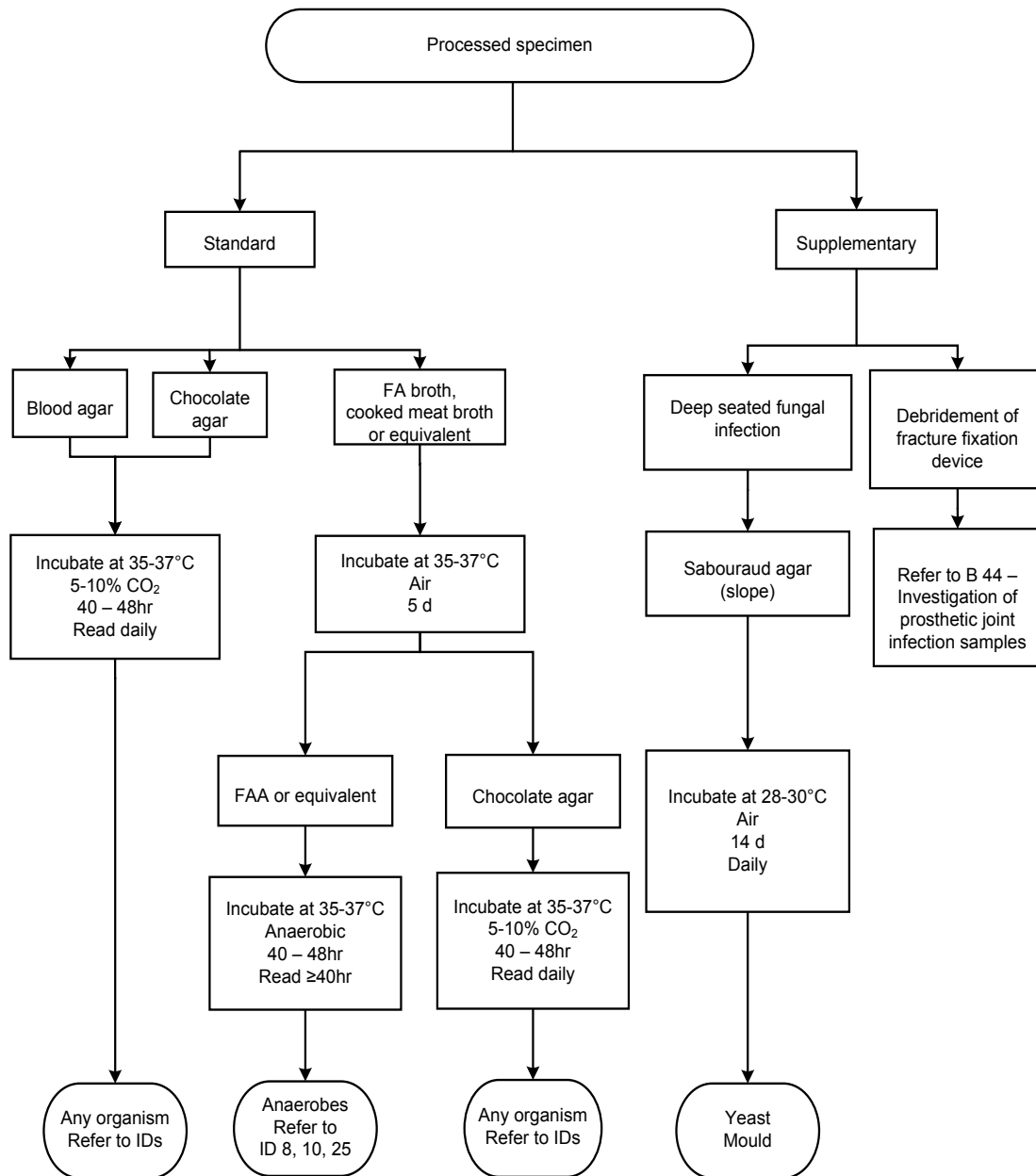
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local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

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)<sup>88,89</sup>, [Wales](#)<sup>90</sup> and [Northern Ireland](#)<sup>91</sup>.

## Appendix 1: Investigation of bone and soft tissue associated with osteomyelitis by culture





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