UK Standards for Microbiology Investigations

Investigation of Bone and Soft Tissue associated with Osteomyelitis

Issued by the Standards Unit, Microbiology Services, PHE

Acknowledgments

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UK Standards for Microbiology Investigations[#]: Status

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- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory service they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
- SMIs also provide commissioners of healthcare services with the 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 essential laboratory methodologies which underpin quality, for example assay validation, quality assurance, and understanding uncertainty of measurement.

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

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

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

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Contents

ISO 9001:2008

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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.

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

Amendment No/Date.	3/dd.mm.yy <tab+enter>
Issue no. discarded.	1.2
Insert Issue no.	#.# <tab+enter>
Section(s) involved.	**Amendment.**

Amendment No/Date.	2/01.08.12
Issue no. discarded.	1.1
Insert Issue no.	1.2
Section(s) involved.	**Amendment.**
Whole document.	Document presented in a new format. The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC[1,2]. Edited for clarity. Reorganisation of [some] text. Minor textual changes.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

Scope of Document

Type of Specimens

Intra-operative samples of bone or bone biopsies sent for clinical diagnosis.

For biopsies and aspirates sent for the investigation of prosthetic joint infections see B 44 – Investigation of prosthetic joint infection samples.

Scope

This SMI describes the processing and microbiological investigation of bone and soft tissue associated with osteomyelitis.

This SMI should be used in conjunction with other SMIs.

Introduction

Osteomyelitis is a progressive infection which results in inflammation of the bone and causes bone destruction, necrosis and deformation[3]. It may be acquired from a contiguous site or haematogenously via the bloodstream, and if left undiagnosed or untreated may lead to chronic infection[4].

Contiguous-focus osteomyelitis may be associated with vascular insufficiency (eg diabetic foot infections) or without vascular insufficiency [4]. In addition it may be associated with an orthopaedic device such as a fracture fixation device or prosthesis. Haematogenous osteomyelitis results from bacteraemic seeding of the bone. In children the commonest site for infection is in the growing ends of long bones. In adults, the spine is the most common site. Risk factors for adult haematogenous osteomyelitis include sickle cell disease, immune deficiencies and intravenous drug use[5].

Gram positive bacteria (*Staphylococcus aureus*, coagulase negative staphylococci, *Enterococcus* species, etc) are most often isolated from bone and soft tissue samples; however 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[6].

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 post-operative wound 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*[7-9]. 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[10,11].

Many of the principles applied to prosthetic joint infection also apply to device-related, contiguous-focus osteomyelitis (B 44 – Investigation of prosthetic joint infection samples)[12]. Where a device is involved, skin flora such as coagulase-negative staphylococci, often regarded as contaminants in the laboratory, are common pathogens. Many devices in long bones and other sites are used to fix fractures; if these become infected, the presentation may be acute, with gross purulence.

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[4].

Diabetic foot infections[13-15]

Diabetic foot infections are responsible for many hospital admissions and a significant number can end up with limb amputation and consequent disability. 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 β-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, sometimes, 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.

Diabetic foot infections are often managed initially by podiatrists; they may perform a deep tissue debridement and send soft tissue for culture, although the presence of osteomyelitis may be indicated on the request card.

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

Acute haematogenous osteomyelitis[3,5,16,17]

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)[5]. In adults the vertebrae are most often affected, however the long bones, pelvis or clavicle may also be affected.[18]

In classical haematogenous osteomyelitis of childhood, the growing ends (metaphyses) of long bones are involved. The commonest organism is *S. aureus*; however β-haemolytic streptococci and HACEK organisms such as *Kingella* species are also important causes[16]. Organisms in the

bloodstream gain access to bone by way of the nutrient artery. They pass though 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[3]. Multiple sites of infection are common in neonates[19]. Between the ages of one and sixteen, *S. aureus*, and *Haemophilus influenzae* type B predominate (although the latter is rare after the age of 5 years and increasingly rare in children under 5 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[20]. 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.

Vertebral osteomyelitis

In adults haematogenous osteomyelitis most commonly involves the spine. 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 immunosupression[21]. 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)[3].

In patients with risk factors, tuberculosis should always be considered; microbiological diagnosis (with or without histology) is required for a definitive diagnosis[22,23]. 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[24,25]. 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 is 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[26].

Sickle cell disease[3,4]

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. *Salmonella* species rarely cause osteomyelitis in patients who are immunocompetent; typically *Salmonella* species are associated with sickle cell anaemia and patients who are immunosuppressed. Infection caused by *Salmonella* species may be due to indirect contamination from an animal host[27,28].

Haemodialysis patients[4]

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[4,29]

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

Chronic osteomyelitis[3,4,18]

Chronic osteomyelitis may be haematogenous or secondary to contiguous-focus infection. Patients typically present with chronic pain and drainage and may have a history of osteomyelitis. 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 ischaemic ulcers associated with diabetes, sickle cell disease and malnutrition. Organisms often associated with chronic osteomyelitis include *S. aureus,* Gram negative bacilli and anaerobic bacteria.

Secondary to contiguous-focus osteomyelitis

In chronic device related infection, organisms may be present in a biofilm that is associated with the device or diseased/necrotic bone. The presence of a biofilm has implications for sampling technique, processing and the interpretation of culture results. Low grade infection eg with skin flora such as coagulase-negative staphylococcus can lead to delayed fracture union or non-union. The organisms may only be present in small numbers and culture often requires broth enrichment[30]. When a patient with a device has debridement, removal and/or exchange of the device, multiple (4-5) samples should be taken using separate instruments. If several are culture positive, whatever the organism(s), this is likely to represent true infection.

Haematogenous

Acute haematogenous osteomyelitis can lead to chronic osteomyelitis characterised by dead areas of bone and sinus tract[31,32]. This condition can fail to respond to treatment and persist for long periods[33]. Infections may recur many years after the first episode[34]

Brodie's abscess[35]

Brodies'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[20,36]. 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[37]. Various genera have been implicated including *Madurella, Acremonium, Pseudoallescheria* and *Actinomadura* species. Fungal mycetomas are termed eumycetomas in contrast to those caused by *Nocardia* species.

Diagnosis [4,15,16,18]

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 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[12]

Samples of bone and soft tissues may be taken from around a prosthetic device, eg a fracture fixation plate or nail. This may be debrided and left *in situ* (debridement and retention) or removed. If the fracture has not united a further internal or external device is usually required. Samples associated with such a device should be processed with the same principles as those associated with prosthetic joint samples (B 44 – Investigation of prosthetic joint infection samples).

The key to processing bone samples is to reduce manipulation to a minimum. Samples can be transferred to the laboratory using routine timescales (ie within hours rather than minutes). In order to reduce the risk of contamination it is desirable to process specimens in a Class 2 microbiological safety cabinet.

Shaking with sterile glass (Ballotini) beads is relatively simple and therefore carries a low risk of contamination. Another method suggested for specimen processing is grinding. However, grinders can break and constitute a health and safety hazard, as can aerosol release from blenders. The problems of heat generated by the grinder (that may be sufficient to damage or kill some microbes), and insufficient strength in the operator to homogenise the tissue in the Griffiths grinding tubes also render this technique non-standardised and with unknown recovery characteristics. The impact of bone and cement fragments on shattering the Griffiths grinding tubes has also not been investigated. Sonication of devices is a means of disrupting bacterial biofilm in vascular and orthopaedic prostheses and has been shown to be useful in the detection of prosthetic joint infection[38-40].

Broth enrichment is important especially in chronic osteomyelitis, device related infections or where the patient has already received antibiotics[30,41]. Broth cultures can become contaminated and so if only one or two samples are taken, a positive culture from one broth is not interpretable[42]. If several (4-5) samples are taken then a definition of a positive microbiological result is easier to create for that patient. In the presence of a device or non-viable bone, any organism cultured in more than one sample may be relevant and should be identified, have extended sensitivities and be reported. Extended sensitivities are particularly important in patients with chronic osteomyelitis, or retained metalwork, where prolonged oral antibiotics are often required.

Management[4,15,16,18]

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[43-45]

Rapid techniques including Nucleic Acid Amplification Tests (NAATs) (eg PCR, 16s rRNA gene PCR, PCR-electrospray ionization (ESI)/MS etc) and matrix-assisted laser desorption ionization time of flight analysis mass spectrometry (MALDI-TOFF/MS) have become available as a means of rapid, sensitive identification of organisms associated with bone and joint infection[46]. PCR methods require the extraction of DNA or RNA from the sample for analysis, whereas MALDI-TOFF differentiates organisms based on their mass/charge spectrum. PCR has been shown to be more sensitive than convention 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 [47,48].

Technical Information/Limitations

Specimen containers[1,2]

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 Specimen Collection, Transport and Storage[1,2]

1.1 Safety considerations[49-60]

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 specimens in sealed plastic bags.

Compliance with postal and transport regulations is essential.

1.2 Achieving optimal conditions

1.2.1 Time between specimen collection and processing

Collect specimens before antimicrobial therapy where possible.

Specimens should be transported and processed as soon as possible.

If possible stop all antibiotics at least 1-2 weeks prior to sampling and consider not giving routine surgical prophylaxis until after sampling. For more information regarding antimicrobial therapy and surgical sampling please refer to B 44 - Investigation of prosthetic joint infection samples.

The volume of the specimen influences the transport time that is acceptable. Larger pieces of bone may maintain the viability of anaerobes for longer[61]. Samples should however not exceed the size of the available CE Marked leak proof containers.

1.2.2 Special considerations to minimise deterioration

If processing is delayed, refrigeration is preferable to storage at ambient temperature[60]. Delays of over 48hr are undesirable.

1.3 Correct specimen type and method of collection

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in Amies transport medium with charcoal[62].

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).

1.4 Adequate quantity and appropriate number of specimens

In 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 specimen collection are dependent on clinical condition of patient.

2 Specimen Processing[1,2]

2.1 Safety considerations[49-60]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[52].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

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

2.2 Test selection

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.

2.3 Appearance

N/A

2.4 Microscopy

2.4.1 Standard

Gram stain (see TP 39 - Staining Procedures)

If sufficient specimen is received prepare as recommended in Section 2.5. 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.

2.5 Culture and investigation

2.5.1 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 0.5 mL) 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.

Supplementary

Fungi and *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species).

2.5.2 Specimen processing

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 5 mL 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 shaking at 250 rpm for 10 minutes in a covered rack on an orbital shaker, or alternatively vortexing for 15 seconds (40 Hz). The diluent for the Ballotini beads and tissues should be Ringer's solution or saline. 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 2 mL.

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.

2.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Standard media	Incubation			Culture s read	Target organism(s)
		Temp °C	Atmos	Time		
‡Osteomyelitis, Bone biopsy, Brodie's abscess, Diabetic foot osteomyelitis, Discitis +For debridement of fracture fixation device refer to B44 – Investigation of prosthetic joint infection.	Blood agar and Chocolate agar	35 - 37	5 - 10% CO_2	40 - 48 hr	Daily	Staphylococci Streptococci Enterobacteriaceae Pseudomonads HACEK group *Nocardia* species*
	Fastidious anaerobic broth, cooked meat broth or equivalent Subculture when cloudy or at day 5 onto plates as below:	35 - 37	air	5 d	N/A	Staphylococci Streptococci Enterobacteriaceae Pseudomonads Anaerobes
Subculture plates	Fastidious anaerobic agar or equivalent	35 - 37	Anaerobic	40 - 48 hr	Daily	Anaerobes
	Chocolate	35 - 37	5 - 10% CO_2	40 - 48 hr	Daily	Any
For these situations, add the following:						
Clinical details/ conditions	**Supplementary media**	**Incubation**			**Culture s read**	**Target organism(s)**
		Temp °C	**Atmos**	**Time**		
Mycetoma,	Sabouraud agar	35 - 37	air	2 - 5 d	≥ 40 hr and 5 d‡	Yeast and Moulds
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.						
*May require incubation for a further 3 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[63,64].						

2.6 Identification

Refer to individual SMIs for organism identification.

2.6.1 Minimum level of identification in the laboratory

Actinomycetes	genus level ID 15 – Identification of anaerobic *Actinomycetes* species
Anaerobes	genus level ID 14 - Identification of non-sporing, non-branching anaerobes ID 8 - Identification of *Clostridium* species ID 25 - Identification of anaerobic Gram-negative rods ID 15 – Identification of anaerobic *Actinomycetes* species
β-haemolytic streptococci	Lancefield group level
Other streptococci	species level
Enterococci	species level
Enterobacteriaceae	species level
Yeast and Moulds	species level
Haemophilus	species level
Pseudomonads	species level
S. aureus	species level
Staphylococci (not S. aureus)	genus level
Mycobacterium species	B 40 - Investigation of specimens for *Mycobacterium* species

Organisms may be further identified if clinically or epidemiologically indicated.

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

2.7 Antimicrobial susceptibility testing

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

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. On Gram-positive these may include a teicoplanin MIC plus antibiotics such as rifampicin, tetracyclines, quinolones, co-trimoxazole, fusidic acid, linezolid, quinupristin/dalfopristin and others.

2.8 Referral to reference laboratories

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

HPA - Specialist and Reference Microbiology Tests and Services

Health Protection Scotland – Reference Laboratories

Belfast Health and Social Care Trust – Laboratory and Mortuary Services

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

2.9 Referral for outbreak investigations

N/A

3 Reporting Procedure

3.1 Microscopy

3.1.1 Gram stain

Report on WBCs and organisms detected.

3.1.2 Microscopy reporting time

Written report: 16 - 72 hr

Urgent microscopy: telephone when available

3.2 Culture

Report any growth.

Report absence of growth.

Also, report results of supplementary investigations.

3.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.[65,66]

4 Notification to PHE

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

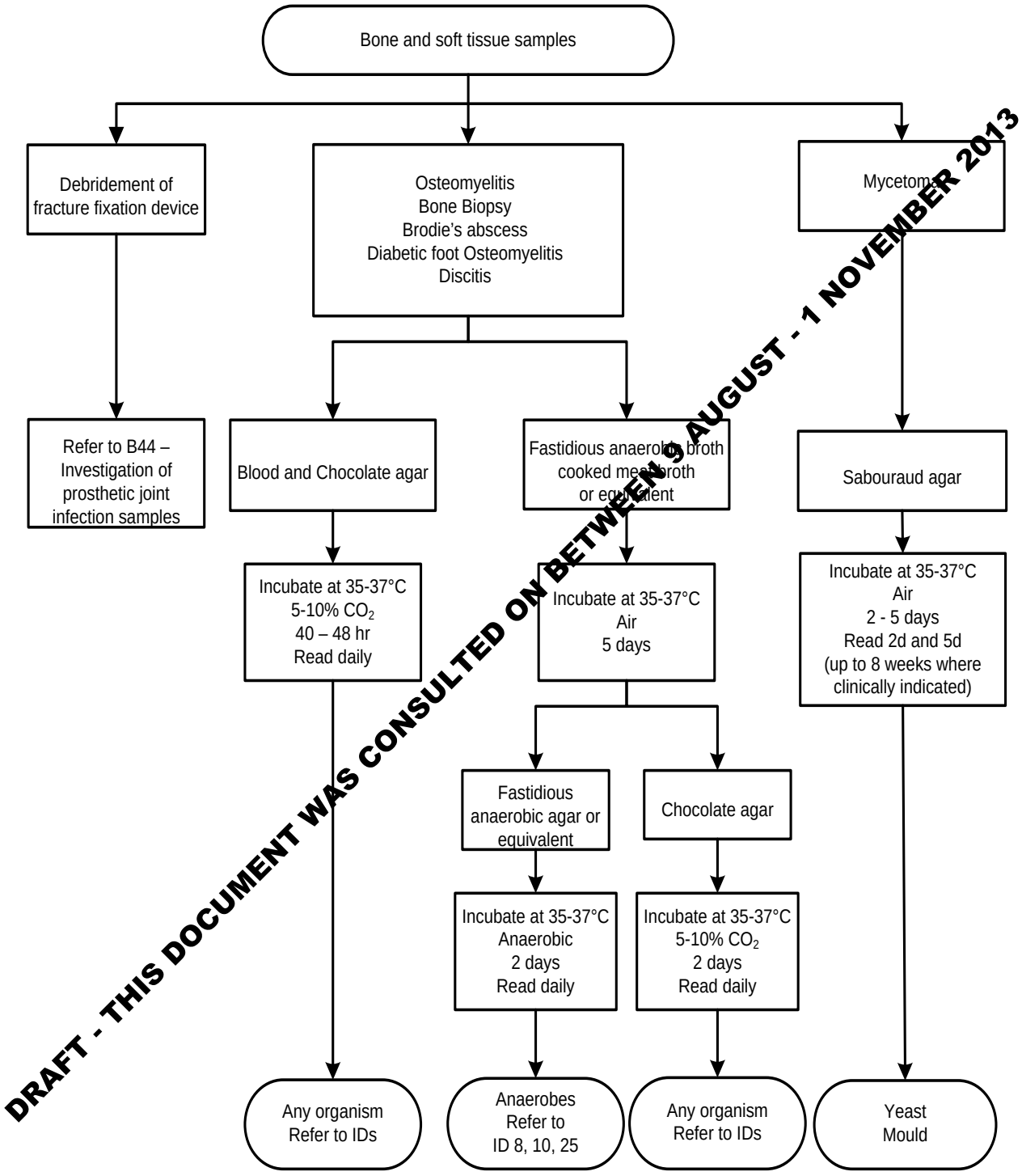
For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of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

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

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

Other arrangements exist in Scotland[67] and Wales[68].

Appendix: Investigation of Bone and Soft Tissue associated with Osteomyelitis Flowchart



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