NATIONAL STANDARD METHOD

INVESTIGATION OF BONE AND SOFT TISSUE ASSOCIATED WITH OSTEOMYELITIS

BSOP 42

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

STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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[1].

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

Suggested citation for this document:

Health Protection Agency (2009). *Investigation of bone and soft tissue associated with osteomyelitis*. National Standard Method BSOP 42 Issue 1.1. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

INDEX

AMENDMENT PROCEDURE

Controlled document reference	BSOP 42
Controlled document title	**Investigation of Bone and Soft Tissue associated with Osteomyelitis**

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
1/ 03.12.09	1	1.1		Front page	SMF logo added
			8 10	Technical Information/Limitations 1.2 Specimen transport and storage	The term “CE marked leak proof container” replaces “sterile leak proof container”; endnote[a] added to clarify the change and referenced to IVD Directive 98/79/EC

INVESTIGATION OF BONE AND SOFT TISSUE ASSOCIATED WITH OSTEOMYELITIS

Type of specimen: 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 BSOP 44 – Investigation of prosthetic joint infection samples).

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the processing and microbiological investigation of bone and associated soft tissue.

INTRODUCTION

Osteomyelitis is inflammation of bone, usually caused by infection. It may be acquired haematogenously (via the bloodstream) or by direct inoculation from a contiguous site (eg a penetrating injury or local ulcer)[2,3]. The latter may be associated with vascular insufficiency (eg in diabetic feet)[4]. In addition osteomyelitis may be associated with an orthopaedic device such as a fracture fixation device or prosthesis. Many of the principles described in the prosthetic joint NSM (BSOP 44 – Investigation of prosthetic joint infection samples) also apply to device related osteomyelitis. In particular, in chronic osteomyelitis and chronic device related infections, organisms may be present in a biofilm that is associated with the device or diseased/dead bone. The presence of a biofilm has implications for sampling technique, processing and the interpretation of culture results.

Haematogenous osteomyelitis

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).

In classical haematogenous osteomyelitis of childhood, the growing ends (metaphyses) of long bones are involved. The commonest organism is *Staphylococcus aureus*, however β-haemolytic streptococci and HACEK organisms are also important causes. 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.

An acute attack of osteomyelitis can lead to chronic osteomyelitis, characterised by dead areas of bone and sinus tracts[5,6]. This condition can fail to respond to treatment and persist for long periods[7]. Infections may recur many years after the first episode[3].

The bacterial species in haematogenous osteomyelitis are usually dependent on the age of the patient. In neonates, Group B streptococci, *Staphylococcus aureus* and *Escherichia coli* cause infection[8]. Multiple sites of infection are common in neonates[9]. 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, aerobic Gram-negative rods occur. *Candida* species[4] may be found when intravenous devices are in use. Usually a single pathogenic organism is isolated but in many cases of chronic osteomyelitis, particularly when associated with wounds and ulcers the disease can be polymicrobial.

Salmonella osteomyelitis

Salmonella species are a rare cause of osteomyelitis in patients who are immunocompetent, but are associated with sickle cell anaemia and patients who are immunosuppressed[10].

Osteomyelitis in intravenous drug users

Septic arthritis, osteomyelitis of the long bones or vertebral discitis are associated with haematogenous infection in intravenous drug users. The commonest organism is *S. aureus*. *Pseudomonas aeruginosa* infections can also be found in the group[11].

Osteomyelitis in haemodialysis patients

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[4].

Brodie's abscess

A specific presentation of osteomyelitis, it is a chronic localised, circumscribed pyogenic intra-medullary 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 and antibiotic therapy are usually curative[12].

Vertebral osteomyelitis

In adults non-device related haematogenous osteomyelitis most commonly involves the spine. Risk factors include older age, a recent intravascular device, haemodialysis, diabetes, IV drug usage (a risk factor for Pseudomonas infection[13]), infection elsewhere and immunocompromise. 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. The commonest organism causing vertebral infections is *S. aureus*[14] followed by streptococci and aerobic Gram-negative rods[15]. In patients with risk factors, tuberculosis should always be considered. In endemic areas *Brucella* species is a common cause of vertebral infection so a travel history should always be sought. Other fastidious Gram-negative rods[15] eg the HACEK group (*Haemophilus* species, *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella* species; (see BSOPID 12 – Identification of Haemophilus species and the HACEK group of organisms) may be occasional causes of vertebral osteomyelitis[16].

Tuberculous osteomyelitis

Extra-pulmonary tuberculosis can occur in many sites including the musculoskeletal system[17,18] . It may occur in any bone or joint. Diagnosis is 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.

Other mycobacteria, such as *Mycobacterium marinum, Mycobacterium avium-intracellulare, Mycobacterium fortuitum* and *Mycobacterium gordonae* have also been associated with bone infections[19], particularly in patients who are immunocompromised.

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

Contiguous 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[2]. Common predisposing factors include surgical reduction and fixation of fractures, prosthetic devices, open fractures and chronic soft tissue infections (see BSOP 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. Puncture wounds of the foot through footwear such as training shoes are particularly associated with osteomyelitis due to *Pseudomonas aeruginosa*[20-23]. This is most commonly seen in children but can also occur in adults[24,25]. Osteomyelitis following human bites, tooth socket infections affecting the mandible, diabetic foot infections and fractures resulting in tissue ischaemia can be caused by anaerobes[26]. *Actinomyces* species are one of the causes of anaerobic osteomyelitis, most commonly involving the jaw.

Device related infections

Where a device is involved, skin flora such as coagulase-negative staphylococci, often regarded as contaminants in the laboratory, are common pathogens. This is similar to prosthetic joint infections and the principles described in BSOP 44 – Investigation of prosthetic joint infection samples apply. 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. Alternatively a low grade infection eg with skin flora such as a 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. 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.

Diabetic foot infections

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 mono-microbial (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 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[27].

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.

Fungal osteomyelitis

Some fungi endemic to certain areas can be associated with osteomyelitis. This includes *Cryptococcus, Blastomyces* and *Sporothrix* species. In patients who are immuno-compromised or those with multiple previous surgical procedures at that site, more common fungi such as *Candida*[4] or *Aspergillus* species can cause osteomyelitis [28]. 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[29]. Various genera have been implicated including *Madurella, Acremonium, Pseudoallescheria* and *Actinomadura* species. Fungal mycetomas are termed eumycetomas in contrast to those cased by *Nocardia* species.

Diagnosis of osteomyelitis

The diagnosis of osteomyelitis usually requires a combination of a full clinical assessment, plain X-rays and possibly further imaging (eg MRI scan, CT scan, and in some situations such as children, ultra-sound) as relevant, 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.

Management of osteomyelitis

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 orally bioavailable 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.

TECHNICAL INFORMATION/LIMITATIONS

In National Standard Methods, the term "CE marked leak proof container" is used to describe containers bearing the CE marking and which are used for the collection and transport of clinical specimens. The requirements of the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1)[30] state that such devices must "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".

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 witheld 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

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 (BSOP 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.

There are no published comparisons or validations of various tissue processing methods in the orthopaedic setting. 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 has been examined in the research setting as a means of disrupting bacterial biofilm in vascular and orthopaedic prostheses. It has not been studied with bone samples.

Broth enrichment is important especially in chronic osteomyelitis, device related infections or where the patient has already received antibiotics[31]. Broth cultures can become contaminated[32] and so if only one or two samples are taken, a positive culture from one broth is not interpretable. 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.

Serology

In cases where specific pathogens such as brucella are suspected, serology should be performed.

Molecular methods

Preliminary assessments of molecular methods applied to tissues to date suggest that the techniques are less sensitive than culture. Comparative validation of culture without liquid enrichment, direct immuno-fluorescence for coagulase-negative staphylococci and propionibacteria and 16S rRNA PCR on material dislodged from resected and transported joint prostheses suggested sensitivities of 22%, 63% and 72% respectively in one study[33]. This could be extrapolated to fixation devices and osteomyelitis. The 16S rRNA PCR may have a clinical role in culture negative cases but in general molecular methods are not yet ready for routine clinical management. Further studies are required to evaluate molecular methods, validated against robust clinical and conventional pathological definitions of infection.

1 SAFETY CONSIDERATIONS[34-44]

1.1 SPECIMEN COLLECTION

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

1.2 SPECIMEN TRANSPORT AND STORAGE

CE Marked leak proof container[a] in a sealed plastic bag.

1.3 SPECIMEN PROCESSING

N/A

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME FOR SPECIMEN COLLECTION

Before antimicrobial therapy where 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 BSOP 44 – Investigation of prosthetic joint infection samples.

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

The specimen should be put in a CE Marked leak proof container[a] in a sealed plastic bag. and sent to the laboratory (direct collection in theatres to a CE Marked leak proof container[a] in a sealed plastic bag with Ringer's or saline solution and Ballotini beads is an option, however microbiology and histology specimen pots can be confused leading to difficulties in processing samples).

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

For intra-operative biopsies collection of multiple (4-5) 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.

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible.

The volume of the specimen influences the transport time that is acceptable. Larger pieces of bone may maintain the viability of anaerobes for longer[45]. Samples should however not exceed the size of the available CE Marked leak proof containers[a].

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48 h are undesirable.

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

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

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

4.3.1 STANDARD

Gram stain (see BSOPTP 39 - Staining Procedures)

If sufficient specimen is received prepare as recommended in Section 4.4. 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.

4.4 CULTURE AND INVESTIGATION

4.4.1 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 (BSOP 40 - Investigation of specimens for Mycobacterium species).

4.4.2 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[a] 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[a] 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 QSOP 52 - Inoculation of culture media).

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

4.4.3 CULTURE MEDIA, CONDITIONS AND ORGANISMS FOR ALL SPECIMENS

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
‡Osteomyelitis, ‡Debridement of fracture fixation device, Bone biopsy, Brodie's abscess, Diabetic foot osteomyelitis, Discitis	Blood and chocolate agar	35 - 37	5 - 10% CO_2	40 - 48 h	Daily	Staphylococci Streptococci Enterobacteriaceae Pseudomonads HACEK group *Nocardia* species*
	Fastidious anaerobic agar	35 - 37	Anaerobic	5 d	Daily	Anaerobes
	*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	35 - 37	Anaerobic	2 d	Daily	Anaerobes
	Chocolate	35 - 37	5 - 10% CO_2	2 d	Daily	Any
Mycetoma, Fungal osteomyelitis	Sabouraud agar	35 - 37	air	2 - 5 d	≥ 40 h: up to 8 weeks	Fungi

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

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

Note: *May require incubation for a further 3 days

4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL OF IDENTIFICATION IN THE LABORATORY

Actinomycetes	genus level BSOPID 15 – Idetification of anaerobic actinomycetes species
Anaerobes	genus level BSOPID 14 - Identification of non-sporing, non-branching anaerobes BSOPID 8 - Identification of Clostridium species BSOPID 25 - Identification of anaerobic Gram-negative rods BSOPID 15 – Idetification of anaerobic actinomycetes species
β-haemolytic streptococci	Lancefield group level
Other streptococci	species level
Enterococci	species level
Enterobacteriaceae	species level
Fungi	species level
Haemophilus	species level
Pseudomonads	species level
S. aureus	species level
Staphylococci (not aureus)	genus level
Mycobacterium	BSOP 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.

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

Report susceptibilities as clinically indicated. See Section 5.3.

5 REPORTING PROCEDURE

5.1 MICROSCOPY

5.1.1 GRAM STAIN

Report on WBCs and organisms detected.

5.1.2 MICROSCOPY REPORTING TIME

Written report:	16 - 72 h
Urgent microscopy:	telephone when available

5.2 CULTURE

Report any growth.

Report absence of growth.

Also, report results of supplementary investigations.

5.2.1 CULTURE REPORTING TIME

Written report: 16 – 72 h stating, if appropriate, that a further report will be issued.

Supplementary investigations: Fungi and *Mycobacterium* species (BSOP 40 - Investigation of specimens for Mycobacterium species).

Clinically urgent results: telephone when available.

5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

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.

See BSOP 45 - Susceptibility Testing for technical details.

6 REPORTING TO THE HPA[46] (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications:

"Reporting to the HPA : A guide for diagnostic Laboratories"

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

Report all isolates of the following:

Mycobacterium species
Brucella species

7 ACKNOWLEDGEMENTS AND CONTACTS

We would like to acknowledge the invaluable help of Dr Bridget Atkins, Dr Ivor Byren and Dr Tony Berendt, Bone Infection Unit, Nuffield Orthopaedic Centre, Oxford, in producing this document.

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa_standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

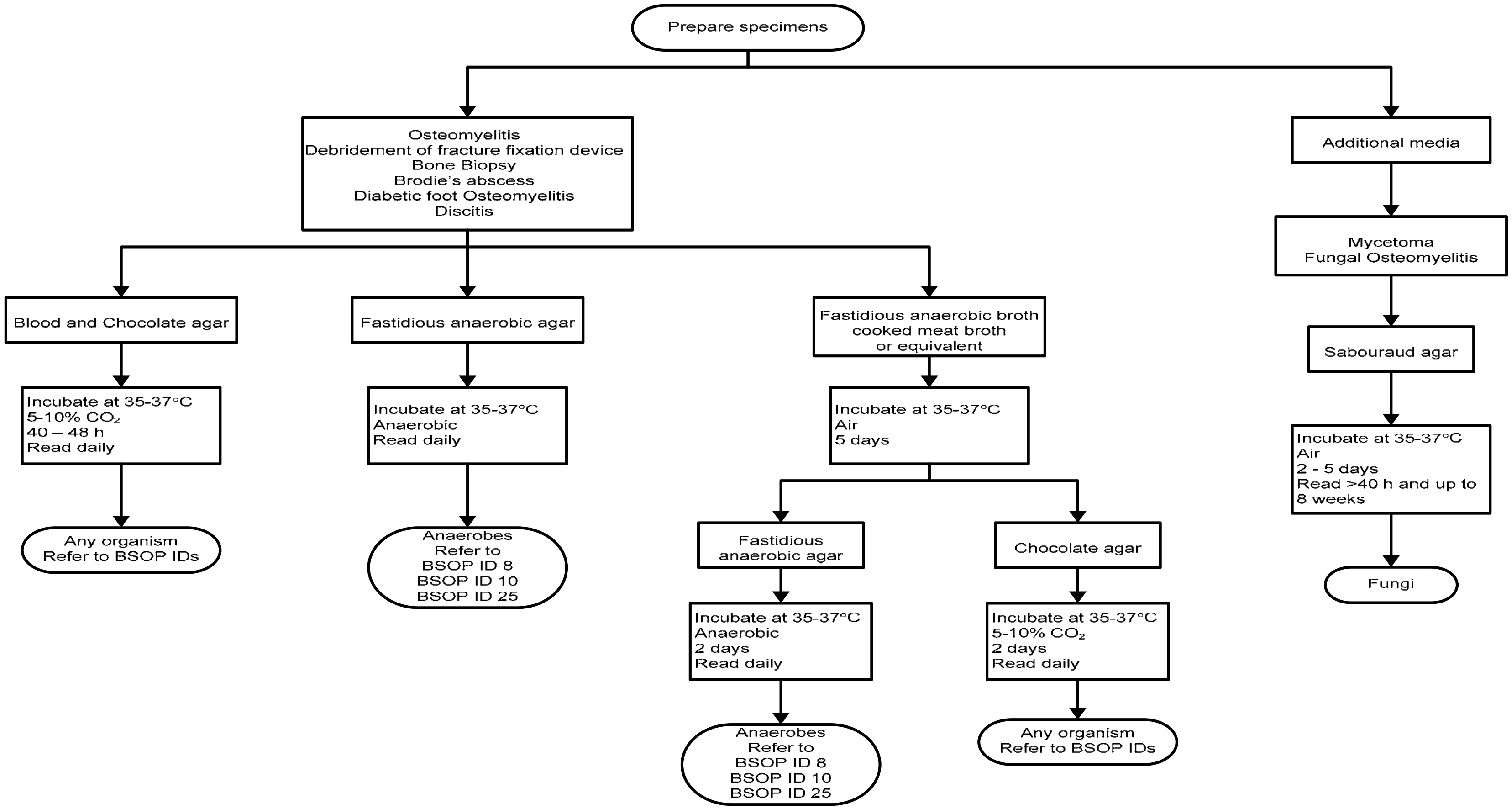
The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

For further information please contact us at:

Standards Unit
Department for Evaluations, Standards and Training
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

APPENDIX


Prepare specimens
Osteomyelitis
Debridement of fracture fixation device
Bone Biopsy
Brodie's abscess
Diabetic foot Osteomyelitis
Discitis
Additional media
Mycetoma
Fungal Osteomyelitis
Blood and Chocolate agar
Fastidious anaerobic agar
Fastidious anaerobic broth
cooked meat broth
or equivalent
Sabouraud agar
Incubate at 35-37°C
5-10% CO_2
40 – 48 h
Read daily
Incubate at 35-37°C
Anaerobic
Read daily
Incubate at 35-37°C
Air
5 days
Incubate at 35-37°C
Air
2 - 5 days
Read >40 h and up to
8 weeks
Any organism
Refer to BSOP IDs
Anaerobes
Refer to
BSOP ID 8
BSOP ID 10
BSOP ID 25
Fastidious
anaerobic agar
Chocolate agar
Fungi
Incubate at 35-37°C
Anaerobic
2 days
Read daily
Incubate at 35-37°C
5-10% CO_2
2 days
Read daily
Anaerobes
Refer to
BSOP ID 8
BSOP ID 10
BSOP ID 25
Any organism
Refer to BSOP IDs

REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.

2. Lew DP, Waldvogel FA. Osteomyelitis. N Engl J Med 1997;999-1007.

3. Waldvogel FA, Papageorgiou PS. Osteomyelitis: the past decade. N Engl J Med 1980;360-70.

4. Mader JT, Calhoun J. Osteomyelitis. In: Madell GL, Dolin R, Bennett JE, editors. Mandell Douglas and Bennett's Principles and Practice of Infectious Diseases. 5th ed. Edinburgh: Churchill Livingstone; 2000. p. 1182-96.

5. Mackowiak PA, Jones SR, Smith JW. Diagnostic value of sinus-tract cultures in chronic osteomyelitis. JAMA 1978;239:2772-5.

6. Mousa HA. Evaluation of sinus-track cultures in chronic bone infection. J Bone Joint Surg Br 1997;79:567-9.

7. Cierny G, Mader JT. Adult chronic osteomyelitis. Orthopaedics 1984;7:1557-64.

8. Ish-Horowicz MR, McIntyre P, Nade S. Bone and Joint infections caused by multiple resistant Staphylococcus aureus in a neonatal intensive care unit. Pediatr Infect Dis 1992;11:82-7.

9. Morrey BF, Peterson HA. Hematogenous pyogenic osteomyelitis in children. Orthop Clin North Am 1975;6:935-51.

10. Tsui HF, Chiu KH, Leung KS. Osteomyelitis of the spine due to Salmonella infection--conservative treatment with quinolone: a case report. Can J Surg 1997;40:48-50.

11. Chandrasekar PH, Narula AP. Bone and joint infections in intravenous drug abusers. Rev Infect Dis 1986;8:904-11.

12. Dunn EC, Singer L. Operative treatment of Brodie's abscess. J Foot Surg 1991;30:443-5.

13. Holzman RS, Bishko F. Osteomyelitis in heroin addicts. Ann Intern Med 1971;75:693-6.

14. Sapico FL, Montgomerie JZ. Pyogenic vertebral osteomyelitis: report of nine cases and review of the literature. Rev Infect Dis 1979;1:754-76.

15. Musher DM, Thorsteinsson SB, Minuth JN, Luchi RJ. Vertebral osteomyelitis. Still a diagnostic pitfall. Arch Intern Med 1976;136:105-10.

16. Farrington M, Eykyn SJ, Walker M, Warren RE. Vertebral osteomyelitis due to coccobacilli of the HB group. Br Med J (Clin Res Ed) 1983;287:1658-60.

17. Wallace R, Cohen AS. Tuberculous arthritis: A report of two cases with review of biopsy and synovial fluid findings. Am J Med 1976;61:277-82.

18. Gorse GJ, Pais MJ, Kusske JA, Cesario TC. Tuberculous spondylitis. A report of six cases and a review of the literature. Medicine (Baltimore) 1983;62:178-93.

19. Marchevsky AM, Damsker B, Green S, Tepper S. The clinicopathological spectrum of non-tuberculous mycobacterial osteoarticular infections. J Bone Joint Surg Am 1985;67:925-9.

20. Rahn KA, Jacobson FS. Pseudomonas osteomyelitis of the metatarsal sesamoid bones. Am J Orthop 1997;26:365-7.

21. Niall DM, Murphy PG, Fogarty EE, Dowling FE, Moore DP. Puncture wound related pseudomonas infections of the foot in children. Ir J Med Sci 1997;166:98-101.

22. Laughlin TJ, Armstrong DG, Caporusso J, Lavery LA. Soft tissue and bone infections from puncture wounds in children. West J Med 1997;166:126-8.

23. Lavery LA, Walker SC, Harkless LB, Felder-Johnson K. Infected puncture wounds in diabetic and nondiabetic adults. Diabetes Care 1995;18:1588-91.

24. Siebert WT, Dewan S, Williams TW, Jr. Case report. Pseudomonas puncture wound osteomyelitis in adults. Am J Med Sci 1982;283:83-8.

25. Gruber HE. Bone and the immune system. Proc Soc Exp Biol Med 1991;197:219-25.

26. Lewis RP, Sutter VL, Finegold SM. Bone infections involving anaerobic bacteria. Medicine (Baltimore) 1978;57:279-305.

27. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2004;39:885-910.

28. Dirschl DR, Almekinders LC. Osteomyelitis. Common causes and treatment recommendations. Drugs 1993;45:29-43.

29. Medical Microbiology L A Guide to Microbial Infections. 15 ed. Edinburgh: Churchill Livingstone; 1997. p. 566

30. IVD Directive. IVD Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. Official Journal of the European Communities 1998;1-37.

31. Dewhurst E, Rawson DM, Steele GC. The use of a model system to compare the efficiency of ultrasound and agitation in the recovery of Bacillus subtilis spores from polymer surfaces. J Appl Bacteriol 1986;61:357-63.

32. Morris AJ, Wilson SJ, Marx CE, Wilson ML, Mirrett S, Reller LB. Clinical impact of bacteria and fungi recovered only from broth cultures. J Clin Microbiol 1995;33:161-5.

33. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, et al. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol 1999;37:3281-90.

34. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Her Majesty's Stationery Office. Norwich. 2004. p. 1-21

35. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. Public Health Laboratory Services (PHLS). London. 1993.

36. HSE L5 Control of Substances Hazardous to Health Regulations. Approved Code of Practice and Guidance. 5th ed. HSE Books; 2002.

37. Health and Safety Executive. 5 Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.

38. Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law. HSE Books. Suffolk. 2002.

39. HSE.Health Services Advisory Committee. Safety in Health Service Laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2. HSE Books. 2003.

40. NHS Estates. Facilities for pathology services. Health building note 15. 2nd. The Stationery Office. London. 2005.

41. British Standards Institution (BSI). Biotechnology- performance criteria for microbiological safety cabinets. BS EN 12469. British Standards Institution. 2000.

42. British Standards Institution (BSI). Microbiological safety cabinets. Part 2: Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. BS 5726:2005. British Standards Institution (BSI). London. 24-3-0005.

43. British Standards Institution (BSI). Microbiological safety cabinets. Part 4: Recommendations for selection, use and maintenance. BS 5726. London. 1992.

44. Advisory Committee on Dangerous Pathogens. The management, design and operation of microbiological containment laboratories. HSE Books. 2001.

45. Holden J. Collection and transport of clinical specimens for anaerobic culture. In: Isenberg HD, editor. Clinical Microbiology Procedures Handbook.Vol 1. Washington D.C.: American Society for Microbiology; 1992. p. 2.2.1-7.

46. Health Protection Agency. Laboratory Reporting to the Health Protection Agency: Guide for Diagnostic Laboratories. 1-5-2008.

---

[a] *The requirements of the EU in vitro Diagnostic Medical Devices Directive[30] (98/79/EC Annex 1 B 2.1) state that such devices must "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".*