

# UK Standards for Microbiology Investigations

## Investigation of Prosthetic Joint Infection Samples



## Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of the 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 <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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# UK Standards for Microbiology Investigations<sup>#</sup>: Status

## Users of SMIs

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

- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory 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 intervention, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

## Involvement of Professional Organisations

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

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

<sup>#</sup> UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

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

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

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

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

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

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

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

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

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## Suggested Citation for this Document

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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](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.	3/dd.mm.yy <tab+enter>
Issue no. discarded.	1.2
Insert Issue no.	dh
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.	<p>Document presented in a new format.</p> <p>The term “CE marked leak proof container” is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to the Directive itself EC.</p> <p>Edited for clarity.</p> <p>Reorganisation of [some] text.</p> <p>Minor textual changes.</p>
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

## Scope of Document

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### Type of Specimen

Prosthetic joint aspirate

Peri-prosthetic biopsy

Intra-operative specimens (debridement and retention or revision surgery)

Prostheses

### Scope

This SMI describes the microbiological investigation of prosthetic joint infection samples. For information regarding bone and tissues samples associated with osteomyelitis refer to [B 42 – Investigation of bone and soft tissue associated with osteomyelitis](#).

This SMI should be used in conjunction with other SMIs.

## Introduction

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Since the earliest hip replacements, pioneered in the UK by Sir John Charnley in the early 1960s, joint replacement (arthroplasty) has become a common procedure. It is done most commonly for osteoarthritis and inflammatory arthropathies such as rheumatoid arthritis. For hip fractures, a hemiarthroplasty is one of the surgical treatment options. Hip and knee replacements are more common than replacements of shoulder, elbow, ankle and interphalangeal joints<sup>1</sup>. Bilateral replacements for osteoarthritis are common in weight bearing joints and multiple joint replacements are common in inflammatory arthritis. Revision surgery is done for joint failure (usually loosening or recurrent dislocation) and the majority are 'aseptic'. Around 15% of revisions are due to 'septic' loosening<sup>2</sup>.

### Risk factors for infection<sup>3</sup>

With modern surgical and anaesthetic techniques, appropriate patient selection, modern prosthesis design, prophylactic antibiotics, good laminar airflow systems in operating theatres and optimum post-operative care, infection rates are now much lower than when joint replacement was first introduced. However there is still a risk associated with each procedure. This is around 1-2% for elective hip and knee replacements and higher for emergency trauma operations eg hemiarthroplasties<sup>4,5</sup>. The risk of infection in a joint replacement is increased by patient co-morbidities, including; the early development of a surgical site infection not apparently involving the prosthesis, a National Nosocomial Infections Surveillance Score of one or two, the presence of malignancy and previous joint arthroplasty<sup>3</sup>. Other co-morbidities such as immunosuppression, diabetes, renal failure, heart or lung disease, smoking and obesity also increase the risk of infection after surgery, as does prolonged post-operative wound drainage and haematoma formation<sup>6</sup>.

### Pathogenesis and microbiology

Organisms may be introduced into the joint during primary implantation surgery or the haematogenous (bloodstream) route<sup>7</sup>. These may cause acute or chronic infections. Fewer organisms are required to establish infection when there is a foreign body *in situ* than otherwise. The most common organism to cause acute infections is *Staphylococcus aureus* (meticillin sensitive or resistant) and in chronic infections either *S. aureus* or coagulase negative staphylococci. It is estimated that up to 30% of *S. aureus* bacteraemias may be associated with septic arthritis in those with pre-existing prosthetic joints<sup>7</sup>. Many other organisms can be acquired by either direct inoculation or the haematogenous route including

other skin flora, streptococci, coliforms, enterococci and rarely anaerobes, mycobacteria or fungi<sup>4,8,9</sup>.

Once infection is established around a prosthetic joint, organisms can form a 'biofilm'<sup>10</sup>. Organisms secrete extracellular substances to produce a complex and sometimes highly organised glycocalyx structure within which they are embedded. In these microbial communities, which may be polymicrobial, some organisms are dividing slowly if at all, and others may even be in a state akin to dormancy. In the microbiological diagnosis of infection, this biofilm may have to be disrupted in order to culture organisms. The "persisters" within the biofilm are very difficult to kill so that infection may not be eradicated without removal of the prosthesis. If it is to be retained, antibiotics with activity against biofilm organisms should be used, but standard antimicrobial sensitivities may not predict the required antimicrobial activity<sup>11</sup>. *In vitro* models testing activity of antimicrobials against biofilm organisms are not at present feasible in routine laboratories.

## Clinical presentation

Prosthetic joint infections can present acutely, with a hot, swollen painful joints. The patient is often febrile and can be clinically septic. Inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are usually raised<sup>11</sup>. This presentation needs to be differentiated from acute inflammatory arthritides such as rheumatoid arthritis, gout, pseudogout and also from an acute haematoma (blood) in the joint. Alternatively, prosthetic joint infections can present chronically. The joint may simply be painful and stiff. There may be evidence for loosening of the prosthesis on X-ray. Inflammatory markers may be slightly raised, but this is non specific<sup>11</sup>. These presentations are often difficult to differentiate from those of mechanical pain or aseptic loosening. The presence of a discharging sinus however, indicates the presence of a deep prosthetic joint infection.

## Diagnosis

In the acute presentation of prosthetic joint infection, in addition to a full clinical assessment of the patient, blood cultures should be taken and a joint aspirate performed. An ultrasound may aid this and will clarify whether there is fluid in the joint itself. Synovial fluid may be visibly purulent or merely turbid. Plain X-rays are performed to look for a fracture or other pathology. In the chronically infected prosthetic joint, the diagnosis is much more difficult. A past history of early post-operative wound infection increases the likelihood of deep infection. Plain X-rays may show loosening but this does not differentiate septic from aseptic loosening. If changes are rapidly progressive over time, infection is more likely. Nuclear radiology may have a role in diagnosis but scans can be non-specific or technically difficult to perform. Magnetic Resonance Imaging (MRI) and computerised tomography (CT) scans are rarely helpful. Inflammatory markers may only be slightly raised and are not specific or sensitive. Sinus cultures are not helpful as organisms cultured do not predict those causing deep infection<sup>12</sup>. A joint aspirate or periprosthetic joint biopsy for microbiology and histology (using ultrasound or other dynamic imaging) are the most specific tests for infection. As organisms may be in a 'sessile' biofilm form (rather than 'planktonic' and loose in the joint fluid) the sensitivity of a joint aspirate can be poor.

## Sample Types

### Percutaneous joint aspiration

This is an important diagnostic test in both acute and chronic prosthetic joint infections. It is performed aseptically, ideally in radiology or in theatres. In acute infections, a Gram stain is useful although a negative result should not rule out the possibility of infection. In chronic infections the sensitivity of a Gram stain is <10%<sup>13,14</sup>. A semi-quantitative white cell count on



the synovial fluid is useful for differentiating inflammatory from non-inflammatory arthritides, however is less useful at differentiating infection from inflammation<sup>2</sup>. A total synovial fluid leucocyte count and differential may be helpful in certain clinical situations (refer to Infectious Diseases Society of America (ISDA) guidelines)<sup>15-17</sup>. Where appropriate synovial fluid should also be examined for crystals. A synovial biopsy may also be considered (see below).

Broth enrichment cultures are important as the patient may have already received antibiotics and in chronic cases the number of free (planktonic) organisms may be very low. In the presence of a joint prosthesis, any organism cultured may be relevant and should be identified, have sensitivity testing performed and be reported. Many chronic infections are due to "skin flora". For this reason differentiating infection from contamination in a sample obtained as an aspirate is difficult. In addition the sensitivity of an aspirate in chronic infection may be poor. A peri-prosthetic tissue biopsy which can include histology could be considered (see below).

### Percutaneously biopsy

A peri-prosthetic biopsy can be obtained under ultrasound or other dynamic imaging, such as fluoroscopy. If the joint is loose, ideally this should be obtained from the bone cement interface or bone prosthesis interface. It has the advantage over needle aspiration alone, that histology, looking for neutrophils, can also be performed if multiple biopsy passes can be performed.

### Intra-operative biopsies

Intra-operative biopsies may be performed in the chronically infected joint either solely as a diagnostic test, as part of a debridement and retention procedure, or when a joint is being revised. Joint revision is a common procedure and usually done for aseptic loosening. However, because infection can be occult, it is advisable to take multiple samples for microbiology and histology in all cases. In some cases, where available, this can be combined with a frozen section to aid decision making<sup>18,19</sup>.

### Sampling

Samples of fluid, pus, synovium, granulation tissue, membrane (the tissue that forms at the bone-cement or bone-prosthesis interface) and any abnormal areas should be taken, in cases where the joint is being removed. Each specimen should be taken with a separate set of instruments, and should be placed into a separate specimen container. Pre-sterilised packs can be produced for this purpose. At this stage a frozen section may also be performed if available and required to decide between one and two stage exchange.

In centres where sonication is available, the prosthesis, or components thereof, can be sent to the laboratory in a sterile watertight container.

### Sample processing

Samples can be transferred to the laboratory using routine timescales (eg within hours rather than minutes). There are no published comparisons or validations of various tissue processing methods in the orthopaedic setting. Shaking with glass beads is relatively simple and therefore carries a low risk of contamination. This method of tissue disruption has been shown experimentally to be superior to shaking in broth alone in the recovery of bacillus spores from polymer surfaces<sup>20</sup>. Results of a study suggest that the use of glass beads in the microbiological examination of intra-operative periprosthetic samples may indeed be a useful addition to conventional culture leading to increased microbiological diagnosis rates with relatively low contamination rates<sup>21</sup>.

Sonication of removed components has been examined as a means of disrupting bacterial biofilm in vascular and orthopaedic prostheses. A considerable number of studies have now

been performed comparing sonication of the prosthesis in a sterile pot to conventional cultures. Several centres have now adopted this as routine practice<sup>1,22-24</sup>.

### Microscopy and Culture

Gram staining in elective revision cases should not be considered for diagnosing infection as it has extremely poor sensitivity. Gram staining does however have a useful role in acute infections<sup>14</sup>. It is important to distinguish between aggregates of ultrasound-dislodged biofilm bacteria from other debris and contaminating bacteria. These can appear as odd single cells or very small groups of cells. A negative Gram stain does not rule out infection. False positive Gram stains associated with periprosthetic infections are rare<sup>13</sup>.

Culture methods should include an enrichment broth. Robertson cooked meat and continuous monitoring blood culture systems (CMBCS) have equivalent sensitivity, and are more sensitive than fastidious anaerobic agar and plates in orthopaedic device related infection<sup>25,26</sup>. In chronic infections, plates may be omitted provided multiple samples are taken and enrichment broths used.

Where plates are also done they can be examined with a plate microscope because small colony variants of staphylococci may be isolated from deep samples. Such small colonies may only become evident on prolonged culture<sup>27</sup>. Thymidine dependent microtrophs usually do not grow on blood agar and have atypical colonial appearance resembling haemophili or streptococci on chocolate agar<sup>28</sup>. The true prevalence and clinical relevance of small colony forms in prosthetic joint infection is unclear.

### Automation

Some laboratories with a significant number of orthopaedic device related samples have opted to use automation using CMBCS to reduce labour and early subculture of culture broths<sup>25,26</sup>.

### Duration of culture

Traditionally orthopaedic samples have been cultured for up to five days. More recently evidence has suggested that incubation for up to 14 days may be necessary to isolate less virulent organisms such as propionibacteria and diptheroids<sup>29,30</sup>. However the methods described omit any early subculture unless broths are cloudy. Visual inspection of broth media is not very accurate; many earlier positive may have been missed. Full automation using CMBCS bottles suggests that such prolonged culture is not necessary and that > 98% of significant results have lagged within 3 days. A pragmatic recommendation is for 5 days of culture using the continuous monitoring blood culture system method.

### Interpretation of results

Defining organisms in separate samples as indistinguishable can be difficult. One or two differences in an extended antibiogram may not always indicate strains from different clonal origins. In addition, infection of prostheses with multiple strains can occur<sup>2</sup>. It is important to perform sensitivity testing on all isolates from all samples as the extended antibiogram is a common and cheap way to identify strains as indistinguishable in multiple cultures and the presence of resistant strains will affect the outcome of therapy.

Organisms can be cultured from 60 - 70% of samples taken from prostheses deemed infected<sup>2</sup>. As the organisms that cause chronic prosthetic joint infection are frequently the same as those that contaminate microbiological samples, interpretation of results is difficult when only one or two samples are taken. When five samples are taken, the false positive rate with two or three samples positive is <5% whereas false positive rates close to 30% are seen with a single positive sample<sup>2</sup>. Growth of an indistinguishable organism from two or more samples is 71% sensitive and 97% specific. Recovery of an indistinguishable organism from three samples is 66% sensitive and 99.6% specific<sup>2</sup>. Obtaining organisms from a single tissue

sample therefore poses significant challenges in interpretation. Even with careful sampling and prolonged cultures, there is still a significant culture negative rate, even when histology is positive. This may be due to sampling error (the distribution of organisms can be patchy), very small numbers of organisms that do not thrive in laboratory culture conditions, an inability to disrupt organisms from the biofilm, unculturable organisms or false positive histology results. Immunofluorescent and molecular studies suggest that, in some cases, there may be organisms present even when conventional cultures are negative<sup>2</sup>.

## Explanted prostheses

Explanted prostheses can be sent for microbiological investigation. They are often difficult to handle unless especially large pots are used (see sonication above) leading to a potentially greater risk of contamination. Some laboratories sonicate the prostheses and culture the sonicate fluid. This can be done in addition to multiple samples but not to replace them. It may reduce the number of tissue samples required to 3-4.

Sonication when used as an addition to conventional culture has been shown to improve the sensitivity of prosthetic joint infection microbiological diagnosis<sup>31,32</sup>. It uses ultrasound to disrupt the bacterial biofilm on the prosthetic material. The sensitivity improvement is most markedly seen in patients on antibiotics within 14 days prior to surgery.

## Rapid techniques

### Serology

Serological techniques used for diagnosis of prosthetic joint infection have been studied in the research setting but have not been found to be of practical clinical use as yet. The problem tends to be with specificity<sup>34</sup>. Measurement of IgM antibodies in patients with vascular graft infections has also been studied although this is not in routine clinical use<sup>35</sup>.

### Molecular methods<sup>36</sup>

#### Nucleic Acid Amplification Techniques (NAATs)

Rapid techniques including PCR, 16S rRNA gene PCR and PCR-electrospray ionization (ESI)/MS have been developed as a means of rapid, sensitive identification of organisms associated with prosthetic joint infection<sup>37</sup>. NAATs methods require the extraction of DNA or RNA from the sample for analysis; these methods have 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 rates of implant related infections<sup>22,38</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 tissues sample, and antibiotic susceptibility information is not available<sup>11,39</sup>.

#### MALDI-TOF Mass Spectrometry<sup>40,41</sup>

Recent developments in identification of bacteria, yeast and fungi include the use of 16S ribosomal protein profiles obtained by Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-TOF) mass spectroscopy<sup>40</sup>. Mass peaks achieved by the test strains are compared to those of known reference strains. It is possible for an organism to be identified from an isolate within a short time frame and it is increasingly being used in laboratories to provide a robust identification system<sup>37</sup>.

## Management

In the absence of radiological or clinical evidence for loosening, some selected patients can be managed with early prosthesis debridement and implant retention. If possible this should be done before the patient receives antibiotics, or at least with a pre-operative aspirate obtained after antibiotics have been withdrawn. In theatre several samples should be taken for microbiology; if the presence of infection is not clear (eg if there is no obvious purulence) histology samples should also be taken. Post-operatively, broad spectrum antibiotics are commenced until the microbiology is clear. When the causative organism(s) are known, therapy can be narrowed according to sensitivities. As organisms are likely to be in biofilm on the retained prosthesis, antibiotics that have activity against organisms in this growth mode should be used where possible. For staphylococcal infection, rifampicin combinations are the most effective<sup>42-44</sup>. Other antibiotics that may be used orally, often in combination with rifampicin (which of course cannot be used in monotherapy because of the risk of development of antimicrobial resistance), are quinolones, fusidic acid, tetracyclines such as doxycycline or minocycline, trimethoprim and co-trimoxazole<sup>45</sup>. Occasionally, linezolid, pristinamycin and other agents may be used<sup>11</sup>. Significant staphylococcal isolates ( $\geq 2$  samples positive) should be tested against all these antimicrobials as well as standard beta-lactams and glycopeptides. Other organisms need appropriate antimicrobial susceptibility testing. The clinician needs as many antimicrobial options as possible as patients often require oral antibiotics for many months and unpredictable intolerance to one or more antimicrobials is common<sup>46,47</sup>.

In cases where a prosthetic joint is chronically painful, functioning poorly and/or loose, an elective revision will be performed. Patients should be off antibiotics for at least 2 weeks. When there is no pre-operative suspicion of infection, revision of the joint in one sitting is done. The effect of a single dose of antibiotic on the sensitivity of microbiological culture is unknown and, where the suspicion of infection is low, timely administration of prophylactic antibiotics is paramount (ie in the 30-60 minutes prior to skin incision)<sup>47</sup>. After opening the joint, multiple (four-five) samples should be taken from different sites for microbiology and equivalent samples taken for histology. The prosthesis may also be sent where sonication is available. It is important for microbiological culture that separate sterile surgical instruments are used for each sample to prevent cross contamination of samples. In some equivocal cases, where available, frozen section for histology can be done, only proceeding to re-implantation if this shows no evidence for infection<sup>19</sup>.

A two stage revision involves the removal of a prosthetic joint and debridement followed by re-implantation. Re-implantation may or may not occur during the same operation. In patients with a known chronically infected joint or one where evidence of infection (purulence) is found intra-operatively, the preferred option in many centres is to remove the joint and do a thorough debridement without immediate re-implantation. This is termed the 'first stage' of a two stage revision. In some centres in selected cases however, one-stage revision is performed even in the presence of infection. Patients should be off antibiotics for at least two weeks. The timing of prophylactic antibiotics is a risk-benefit decision. It may be reasonable to give antibiotics after sampling, especially if a new joint will not be re-implanted. Even if a one-stage is performed, delaying antibiotics until after sampling will still allow high levels prior to insertion of new prosthesis. Again, multiple samples should be taken, as described above. In some cases (and in all knees) an antibiotic-loaded cement spacer is put in to protect the joint integrity and avoid impaction of debrided bone ends. Commercially available cements contain antibiotics such as gentamicin<sup>48</sup>. Post-operatively, patients generally receive broad spectrum antibiotics until microbiological results are available. Definitive therapy is usually for several weeks until there is good evidence that the wound is healed and inflammatory markers have normalised. If re-implantation is planned this is performed at this stage or any time afterwards.

## Technical Information/Limitations

### Specimen containers<sup>49,50</sup>

SMLs 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”.

Sonication containers are available. See below.

### Sonication

Gram-positive bacteria have been found to be resistant to the effect of ultrasound; Gram negative organisms may be more susceptible<sup>24</sup>. The effect of sonication on fungi and *Mycobacterium* species is unknown.

There may be the potential for contamination of sonication fluid during collection or specimen processing. Particular care needs to be taken during opening and closing the container lid, to ensure that no contact is made with inner surface of the lid. Contamination is usually indicated by low counts of environmental bacteria.

## 1 Specimen Collection, Transport and Storage<sup>49,50</sup>

### 1.1 Safety considerations<sup>51-62</sup>

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

Use aseptic technique.

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.

#### 1.2.2 Special considerations to minimise deterioration

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>62</sup>.

Delays of over 48hr are undesirable.

### 1.3 Correct specimen type and method of collection

Swabs are to be discouraged. However, if sent, swabs for bacterial and fungal culture should then be placed in Amies transport medium with charcoal<sup>63</sup>.

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



## 1.4 Adequate quantity and appropriate number of specimens

For aspirates and radiologically guided biopsies, it is usually only possible to send one sample to microbiology. In theatres, multiple (four to five samples) should be taken using separate instruments for microbiology. An equivalent set of samples should be taken for histology.

Specimen size should approximate to 1 mL.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

## 2 Specimen Processing<sup>49,50</sup>

### 2.1 Safety considerations<sup>51-62</sup>

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>54</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.

### 2.2 Test selection

N/A

### 2.3 Appearance

N/A

### 2.4 Microscopy

#### 2.4.1 Standard

Gram stain (refer to [TP 39 - Staining Procedures](#))

This is an insensitive procedure and not recommended for the pre or intra-operative diagnosis of chronic prosthetic joint infection.

It does however have a role in acute prosthetic joint infection especially on a purulent aspirate or surgical pus. It is important to distinguish between aggregates of ultrasound-dislodged biofilm bacteria from other debris and contaminating bacteria. These can appear as odd single cells or very small groups of cells. A negative Gram stain does not rule out infection.

### 2.5 Culture and investigation

#### 2.5.1 Pre-treatment<sup>20,21</sup>

The objective should be to minimise the manipulation on the number of times any container is opened and resulting exposure of the operative sample to contamination.

It may be possible in units with high workloads of this specimen type to arrange provision and use of CE Marked leak proof container with approximately 10 glass beads and 5 mL Ringer's or normal saline to the operating theatre. It is not uncommon, however, for microbiology and histology specimen pots to be confused leading to difficulties in processing samples. Transfer of biopsies in theatres may diminish the risk of contamination during laboratory processing. In such circumstances homogenisation could be performed in the original container.

Alternatively, samples may be sent to the laboratory in CE Marked leak proof container in a sealed plastic bag with no glass beads. Glass beads and Ringer's or saline can be added in the laboratory, maintaining asepsis diligently. Clean air provision may be desirable.

Homogenisation with glass 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 (40Hz).

The diluent for the glass beads and tissues should be Ringer's or saline. Sterile molecular grade water and new universal containers should be used if direct PCR assays are planned. The volume used in the latter case should not exceed 2 mL to maintain assay sensitivity. As an alternative to enrichment broth, samples may be cultured in an automatic continuous monitoring blood culture system for up to 5 days. Only subculture bottles which flag positive; a terminal subculture at five days is not required.

## 2.5.2 Specimen processing

### Soft tissue homogenate

Inoculate plates and broth after homogenisation. Inoculate each agar plate (if used) with a drop of the solution using a sterile pipette (see [Q 5 - Inoculation of Culture Media for Bacteriology](#)). In addition, place some of the solution into an enrichment broth. If mycobacterial cultures are required this solution can then be used to inoculate mycobacterial cultures. This is best done 24 hr after the primary plates have been examined once, to decide if recontamination of the sample is required.

Incubate the enrichment broth for a further 14 days, examining daily for turbidity. Subculture if cloudy but otherwise perform a terminal subculture at 14 days. As an alternative to enrichment broth, samples may be cultured in an automatic continuous monitoring blood culture system for 5 days. Only subculture bottles which flag positive; a terminal subculture at five days is not required.

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

If done, primary plates should be examined with a plate microscope for small-colony variants<sup>27,64</sup>. Care should be taken to distinguish small tissue fragments on the plate from small colonies. Small colony variants are often thymidine-dependent, at least if the patient has received co-trimoxazole. Such isolates may not grow well on horse blood agar due to partial lysis and release of thymidine phosphokinase from the red cells. The heating process used to produce chocolate agar destroys thymidine phosphokinase.

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## 2.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
All clinical conditions  (Primary plates may not be needed in elective revisions, in high volume units and skilled multiple site sampling.)	Blood agar and Chocolate agar	35 - 37	5 - 10% CO <sub>2</sub>	40-48 hr	Daily	Staphylococci Streptococci Enterococci Enterobacteriaceae Fastidious Gram negatives Pseudomonads Yeast Mould
	* Cooked meat broth or equivalent. Subculture when cloudy or at day 14 on plates as below or **blood culture for CMBCS. Subculture when flags positive on plates as below	35 - 37	Air	14 d for Cooked meat broth or up to 5 d for CMBCS	N/A	Any
Subculture plates	Blood agar	35-37	Anaerobic	40-48 hr	Daily	Any Anaerobes
	Chocolate agar	35 - 37	5 - 10 % CO <sub>2</sub>	40-48 hr	Daily	Any
	Labouraud' s agar	30	Air	14 d	Daily	Yeast and Mould
<p>Always consider other organisms such as <i>Mycobacterium</i> species (<a href="#">B 40 - Investigation of specimens for <i>Mycobacterium</i> species</a>), fungi and actinomycetes.</p> <p>* Subcultures should be performed when the broth looks cloudy, terminal subcultures should be performed at 14 days. Blood culture subcultures should be performed when the bottle flags positive. A terminal subculture is not required.</p>						

## 2.6 Identification

Refer to individual SMLs for organism identification.

### 2.6.1 Minimum level of identification in the laboratory

<a href="#">Actinomycetes</a>	genus level <a href="#">ID 15 – Identification of anaerobic <i>Actinomycetes</i> species</a>
<a href="#">Anaerobes</a>	genus level <a href="#">ID 14 - Identification of non-sporing, non-branching anaerobes</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="#">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 Mould	species level
<a href="#">Haemophilus species</a>	species level
<a href="#">Pseudomonads</a>	species level
<a href="#">S. aureus</a>	species level
<a href="#">Staphylococci</a> (not aureus)	to coagulase negative Staphylococci
<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.

Refer to individual SMLs for organism identification.

**Note:** No organism should be considered to be a contaminant until cultures on all samples are concluded. Identification to species level and/or an extended antibiogram is normally necessary to detect whether isolates from multiple samples are indistinguishable.

**Note:** Laboratories should save all samples and isolates for at least 2 weeks in case further work (unusual organisms, molecular studies or further sensitivities) is required.

## 2.7 Antimicrobial susceptibility testing

Extensive antibiograms (including rifampicin) are required<sup>16</sup>.

Refer to British Society for Antimicrobial Chemotherapy (BSAC) guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

## 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

N/A

### 3.2 Culture

Report all organisms.

Report absence of growth.

Also, report results of supplementary investigations.

Intra-operative samples Interpretation: Two or more samples with an indistinguishable organism are a positive microbiology result.

#### 3.2.1 Culture Reporting Time

Written report: 16 hr –14 days stating, if appropriate, that a further report will be issued.

Supplementary investigations: [B 39 - Investigation of dermatological specimens for superficial mycoses](#), and [B 40 - Investigation of specimens for Mycobacterium species](#).

Clinically urgent results: telephone when available.

### 3.4 Antimicrobial susceptibility testing

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

## 4 Notification to PHE<sup>65,66</sup>

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



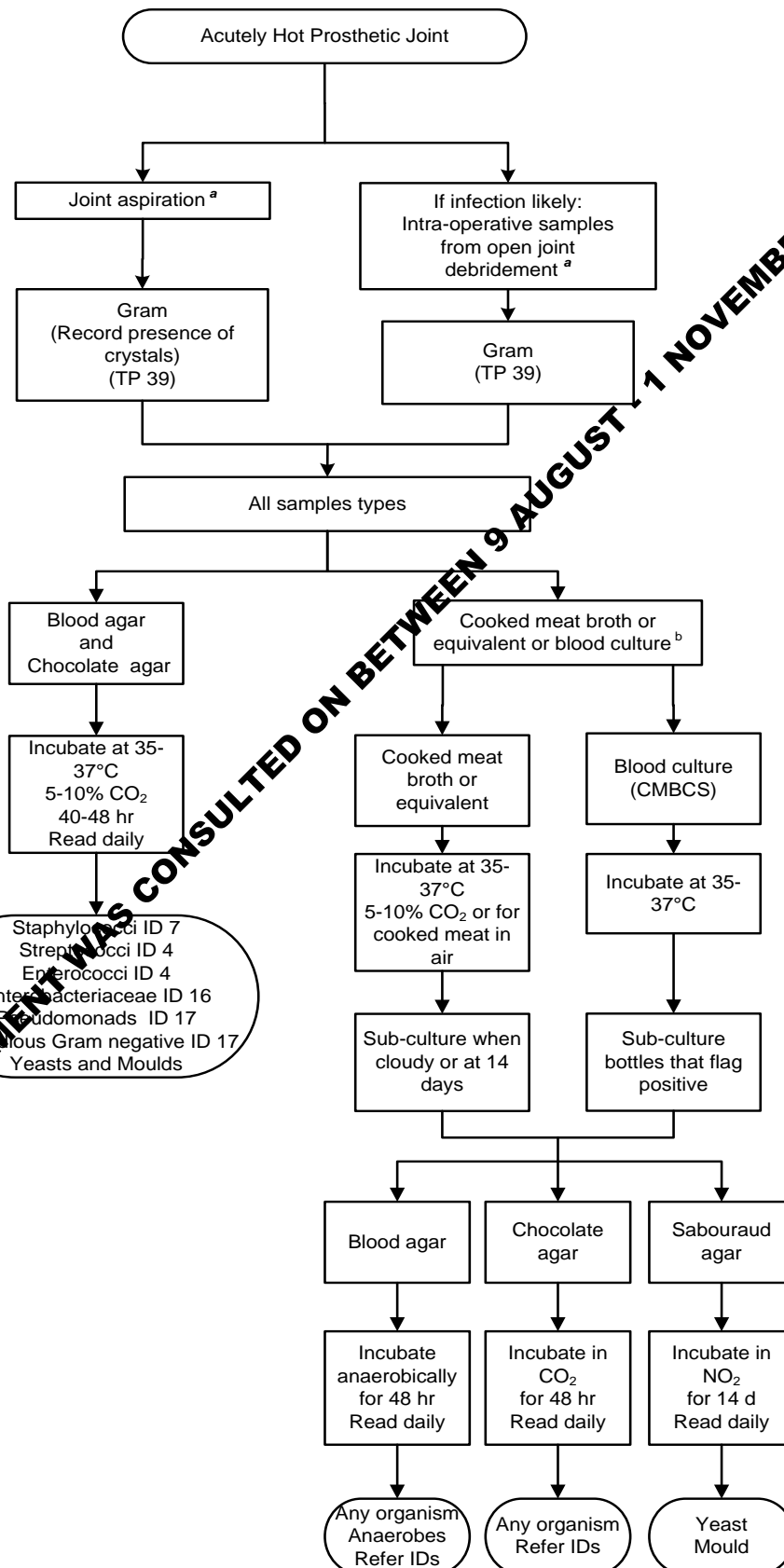
Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

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

Other arrangements exist in Scotland<sup>67</sup> and Wales<sup>68</sup>.

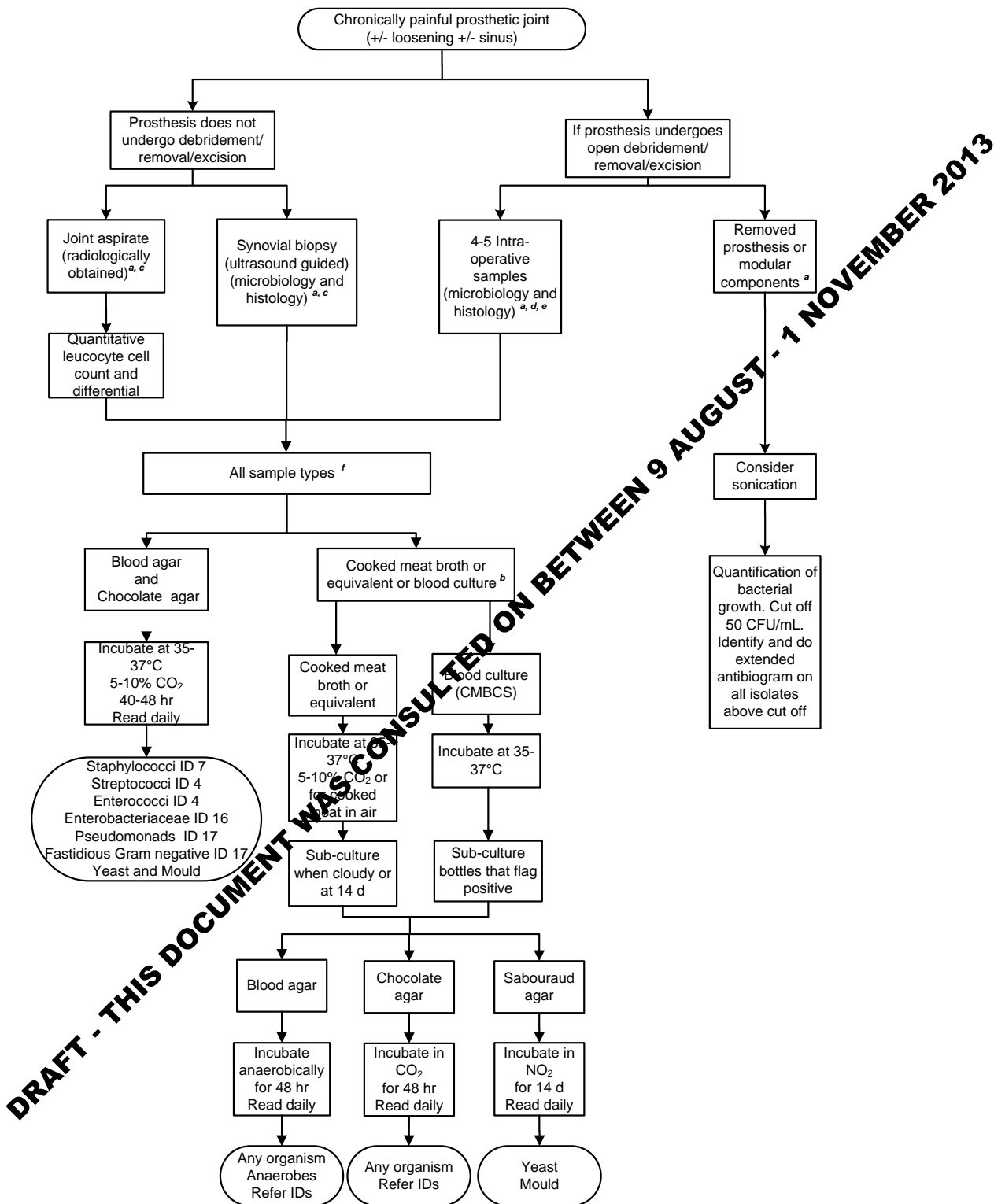
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# Appendix 1: Investigation of the Acutely Hot Prosthetic Joint



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# Appendix 2: Investigation for Chronic Prosthetic Joint infection



## Footnotes

- a) Laboratories should save all samples and isolates for at least 2 weeks in case further work (unusual organisms, molecular studies or further sensitivities) is required.
- b) As an alternative to enrichment broth, samples may be cultured in an automatic continuous monitoring blood culture system (CMBCS) for up to 5 days. Terminal subculture is not required.
- c) These tests may not be required if proceeding straight to revision/removal.
- d) Use separate sterile instruments (from resection arthroplasty, first of a second stage revision or first stage revision).
- e) Interpretation: Two or more samples with an indistinguishable organism are a positive microbiology result.
- f) Mycobacterial cultures if any clinical suspicion eg ethnic origin, plus previous unexplained culture negative samples, granulomas on histology, chest x-ray findings, and previous history of TB (see [B 40 – Investigation of specimens for \*Mycobacterium\* species](#)).

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