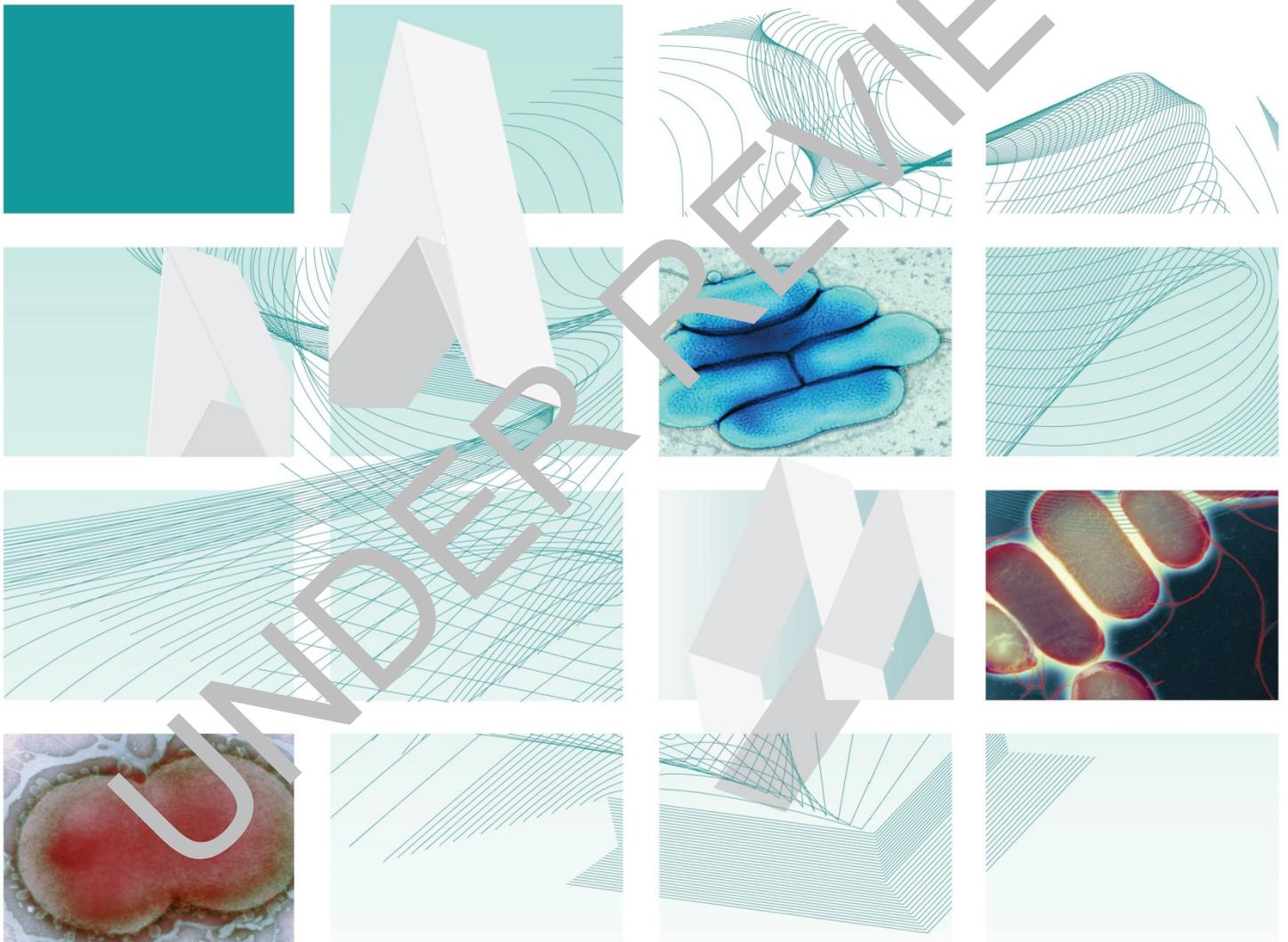




# UK Standards for Microbiology Investigations

## Investigation of Prosthetic Joint Infection Samples



## Acknowledgments

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For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## 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.	3/27.05.14
Issue no. discarded.	1.2
Insert Issue no.	1.3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	2/01.08.12
Issue no. discarded.	1.1
Insert Issue no.	1.2
<b>Section(s) involved</b>	<b>Amendment</b>
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	Reorganised. Previous numbering changed.

collection, transport, storage and processing.	
References.	Some references updated.

UNDER REVIEW

## UK SMI<sup>#</sup>: Scope and Purpose

### Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

### Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post-analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

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

### Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at [http://www.hpa.org.uk/SMI\\_Partnerships](http://www.hpa.org.uk/SMI_Partnerships). Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of producing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two-way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

### Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory

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

investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

## Patient and Public Involvement

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

## Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives

<http://www.hpa.org.uk/webc/HPAwebFile/HPAwebContent/1/2/17133470313>.

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

## Legal Statement

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

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

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## 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

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This SMI describes the processing and bacteriological investigation of prosthetic joint infections. For information on bone samples 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 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 early surgical treatment options. Hip and knee replacements are more common than replacements of shoulder, elbow, ankle and interphalangeal joints. Spinal disc replacements are a very recent introduction, which is still at the developmental stage. Bilateral replacements for osteoarthritis are common in weight bearing joints and multiple joint replacements are common in inflammatory arthritis. Multiple replacements carry the important implication that local symptoms and localised microbiological diagnosis of infection are critical in determining which joint is subjected to revision arthroplasty. It is important to note that other complications are more common causes of the need for joint revision than infection and this has been true since the earliest days of arthroplasty<sup>1</sup>. In one series only some 14% of revision surgery was performed for infection<sup>2</sup>.

With modern surgical and anaesthetic techniques, appropriate patient selection, modern prostheses design, prophylactic antibiotics, ultraclean laminar airflow in operating theatre and good post-operative care, infection rates are now much lower than when joint replacement was first introduced. However, there is still a finite risk associated with each procedure. This is around 1% for elective hip and knee replacements and 4% for emergency hemiarthroplasties. 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>4</sup>.

Organisms may be introduced into the joint, establishing acute or chronic infection, during primary implantation surgery or the haematogenous (bloodstream) route. Fewer organisms are required to establish infection when there is a foreign body *in situ* than otherwise<sup>5</sup>. It is estimated that up to 30% of *S. aureus* bacteraemias are associated with septic arthritis in those with pre-existing prosthetic joints<sup>6</sup>. 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. 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>7-9</sup>. This means that any organism cultured from a sample associated with a prosthetic joint or other orthopaedic device could be significant. It is for this reason that multiple samples should be taken.

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. *In vitro* models testing activity of antimicrobials against biofilm organisms are not at present feasible in routine laboratories.

Prosthetic joint infections can present acutely, usually with a hot, swollen painful joint. 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 markedly raised. 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. These presentations are often difficult to differentiate from those of mechanical pain or aseptic loosening, whereas the presence of a discharging sinus indicates the presence of a deep prosthetic joint infection. Ultimately many painful, loose prostheses require surgical revision (exchange). Of patients undergoing elective revision around 14% are found to be infected<sup>2</sup>.

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 if possible. Synovial fluid may be visibly purulent or merely turbid. Plain X-rays are performed to rule out fracture and to look for evidence of infection. 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. MRI and CT 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>11</sup>. A joint aspirate or periprosthetic joint biopsy for microbiology and histology 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, however, can be poor. A joint aspirate can be performed on the ward, in radiology departments or in

theatre, at the discretion of the orthopaedic surgeon who should always be involved in management decisions.

In the absence of radiological or clinical evidence for loosening and with a short duration of symptoms, 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 off antibiotics. In theatre, several samples should be taken for microbiology and if the presence of infection is not clear (eg if there is no obvious purulence), also for histology. 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 may be the most effective<sup>12-14</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, and co-trimoxazole<sup>15</sup>. Occasionally linezolid, quinupristin-dalfopristin and other agents may be used.

In cases where a prosthetic joint is chronically painful and loose but the presence of infection is not known, an elective revision may be performed. When there is no pre-operative suspicion of infection, revision of the joint in one sitting is recommended. After opening the joint, multiple (four-five) samples should be taken from different sites for microbiology and equivalent samples taken for histology. 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. When a tourniquet is used, antibiotics should be administered before inflation. The effect of a single dose of antibiotic on the sensitivity of microbiological culture is unknown. It is important for microbiological culture that separate 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.

In patients with a chronically infected joint, either discovered at routine revision, or diagnosed by the presence of a sinus or microbiological tests, the preferred option in many centres is to remove the joint and do a thorough debridement without immediate re-implantation. In some centres, one-stage revision is performed even in the presence of infection. Again, multiple samples should be taken, as described above. In some cases (especially in infected knee replacements) 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 or tobramycin. 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

### Limitations of UK SMIs

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

### Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

### Specimen Containers<sup>16,17</sup>

SMIs use the term, "CE marked leak proof container," to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes."

### Percutaneous Joint Aspiration

This is an important diagnostic test in both acute and chronic prosthetic joint infections. It is important that this 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. 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>. In the latter, crystals should be searched for in the synovial fluid. A quantitative and differential white cell count may be helpful in patients with underlying osteoarthritis. In one study, a leukocyte count of  $>1.7 \times 10^3/\mu\text{L}$  had a sensitivity of 94% and specificity of 88% for diagnosing prosthetic infection compared with aseptic loosening. The authors however excluded all patients with an underlying inflammatory arthropathy<sup>18</sup>.

Broad 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 is poor. A peri-prosthetic tissue biopsy which can include histology should be considered (see below).

## Percutaneous 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>19</sup>.

Samples should be taken early in the procedure, just prior to administering prophylactic antibiotics, where infection is likely and/or a microbiological diagnosis is likely to significantly affect outcome. When a tourniquet is used, antibiotics should be administered before inflation. Samples of fluid, pus, synovium, granulation tissue and any abnormal areas should be taken, particularly from the peri-prosthetic 'membranes', (the tissue that forms at the bone-cement or bone-prosthesis interfaces), 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.

Especially in cases with suspected infection, an adequate debridement is crucial. If the prosthesis is to be retained, this will only involve removal of dead tissue, loose cement or bone graft, drainage of pus, and exchange of any modular components as clinically determined. If the prosthesis is being removed, this must also include all abnormal tissue areas, dead bone, cement (including the cement restrictor from replacement hips) and other foreign material.

Following debridement the wound can be closed over drains, or in the case of a one-stage revision, may be covered or temporarily closed while the surgeon re-scrubs and prophylactic antibiotics are given prior to re-implantation of a new prosthesis.

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 Ballotini 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>.

Sonication has been examined in the research setting as a means of disrupting bacterial biofilm in vascular and orthopaedic prostheses. Clinical studies of sonication in orthopaedics have, until recently, been fraught with practical difficulties and specimen bag leakage. A recent study appears to have overcome the risk of leakage by using specimen pots large enough to accommodate the prosthesis; however, sonication of orthopaedic samples remains a technique for the clinical research setting at present<sup>21</sup>.

Gram staining in elective revision cases has extremely poor sensitivity. It has a useful role in acute infections. Organisms can be cultured from 60-70% of samples taken from prostheses deemed to be infected (using histology as a surrogate criterion standard)<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. At least four to five samples are recommended. 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. 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. 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. Immuno-fluorescent and molecular studies suggest that, in some cases, there may be organisms present even when conventional cultures are negative.

The culture methodology that has been validated by comparison with histology involves liquid culture and prolonged incubation on both primary and subculture plates<sup>2</sup>. No comparative culture methodologies have been evaluated, and it is not clear which components of the described methodology are critical. It may not be important in elective revisions to include plates provided multiple sites are sampled and put into broth media. Work with *S. epidermidis* and vascular grafts suggests that liquid enrichment is as important as sonication or grinding<sup>22</sup>. Exclusion of contaminants during operative and laboratory processing is important.

Examination of culture plates with a plate microscope may be important because small colony variants of staphylococci may be isolated from deep samples. Such small colony variants may emerge on vancomycin therapy, although this particular description relates to catheter associated infections<sup>23</sup>. Gentamicin and co-trimoxazole use are more frequently associated with emergence of small colony auxotrophs for thymidine, menadione, or haemin<sup>24</sup>. Such small colonies may only become evident on prolonged culture<sup>25</sup>. Thymidine dependent auxotrophs usually do not grow on blood agar and have atypical colonial appearance resembling haemophilus or streptococci on chocolate agar<sup>26</sup>. The true prevalence of small colony forms in prosthetic joint infection in cemented prostheses is unclear. The organisms may be present in areas near the prosthesis with low concentrations of antibiotic diffusing from the cement.

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 presence of resistant strains will affect the outcome of therapy, and the extended antibiogram is a common and cheap way to identify strains as indistinguishable in multiple cultures.

## 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.

## Serological Techniques

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>27</sup>. Measurement of IgM antibodies in patients with vascular graft infections has also been studied although this is not in routine clinical use<sup>28</sup>.

## 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 rDNA PCR on material lodged from resected and transported prostheses in one study suggested sensitivities of 22%, 63% and 72% respectively<sup>29</sup>. PCR for 16S rDNA 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, and validate them against robust clinical and conventional pathological definitions of infection.

# 1 Safety Considerations<sup>16,17,30-44</sup>

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## 1.1 Specimen Collection, Transport and Storage<sup>16,17,30-33</sup>

Use aseptic technique.

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

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

Compliance with postal and transport regulations is essential.

## 1.2 Specimen Processing<sup>16,17,30-44</sup>

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>36</sup>.

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

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

# 2 Specimen Collection

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## 2.1 Type of Specimens

Prosthetic joint aspirate, peri-prosthetic biopsy, intra-operative specimens (debridement and retention or revision surgery), prostheses

## 2.2 Optimal Time and Method of Collection<sup>45</sup>

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible<sup>45</sup>.

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium<sup>46-50</sup>.

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

## 2.3 Adequate Quantity and Appropriate Number of Specimens<sup>45</sup>

Specimen size should approximate to 1mL.

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

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.

## 3 Specimen Transport and Storage<sup>16,17</sup>

### 3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible<sup>45</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>45</sup>.

## 4 Specimen Processing/Procedure<sup>16,17</sup>

### 4.1 Test Selection

N/A

### 4.2 Appearance

N/A

### 4.3 Sample Preparation

For safety considerations refer to Section 1.2.

### 4.4 Microscopy

Refer to [TP 39 - Staining Procedures](#).

#### Gram stain

**Note:** 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.

### 4.5 Culture and Investigation

#### 4.5.1 Pre-treatment

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 Ballotini beads and 5mL 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 Ballotini beads. Ballotini beads and Ringer's/normal saline can be added in the laboratory, maintaining asepsis diligently. Clean air provision may be desirable. Homogenisation with Ballotini beads can be

performed by shaking at 250 rpm for 10min in a covered rack on an orbital shaker or, alternatively, vortexing for 15sec (40Hz).

The diluent for the Ballotini beads and tissues should be Ringer's/normal 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 2mL to maintain assay sensitivity.

## 4.5.2 Specimen processing

### Soft tissue homogenate

Inoculate plates and broth after homogenisation. Inoculate each agar plate 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 24hr after the primary plates have been examined once, to decide if decontamination of the sample is required.

Incubate the enrichment broth for a further five days, examining daily for turbidity. Subculture if cloudy but otherwise perform a terminal subculture at five days. See table 4.5.3 for primary and subculture plates, broths, atmospheres and duration of cultures.

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

Primary plates should be examined with a plate microscope for small-colony variants. 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. Choculating destroys thymidine phosphokinase.

### 4.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp. °C	Atmos.	Time		
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>	2d	Daily	Staphylococci Streptococci Enterococci Enterobacteriaceae Fastidious Gram negatives Pseudomonas Fungi
	Fastidious anaerobic agar	35-37	Anaerobic	5d	48hr	Anaerobes
	*Fastidious anaerobic broth, cooked meat broth or equivalent Subculture when cloudy or at day five on plates as below	35-37	5-10% CO <sub>2</sub> or for Cooked meats – in air	5d	N/A	Any
	Subculture plates: Blood agar – incubated anaerobically for 48hr	35-37	Anaerobic	2d	Daily	Any
	Sabouraud's agar	30	Air	14d		
Chocolate agar incubated for 48hr	35-37	5-10% CO <sub>2</sub>	2d	Daily	Any	
* Subcultures should be performed when the broth looks cloudy, terminal subcultures should be performed at five days.						

Other organisms for consideration: Mycobacterium species, fungi and actinomycetes.

## 4.6 Identification

Refer to individual SMIs for organism identification.

### 4.6.1 Minimum level of identification in the laboratory

<a href="#">Actinomycetes</a>	genus level <a href="#">ID 15 – Identification of Anaerobic Actinomyces species</a>
<a href="#">Anaerobes</a>	genus level

	<a href="#">ID 14 - Identification of Anaerobic cocci</a> <a href="#">ID 8 - Identification of <i>Clostridium</i> species</a> <a href="#">ID 25 - Identification of Anaerobic Gram Negative rods</a>
<a href="#">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
<a href="#">Fungi</a>	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)	usually genus level
<a href="#">Mycobacterium species</a>	<a href="#">B 40 - Investigation of Specimens for <i>Mycobacterium</i> species</a>

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

Note 1: Subculture after homogenisation, which is likely to generate aerosols, must be performed in a safety cabinet<sup>40</sup>.

Note 2: 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 3: Organisms should be stored until such a time as the clinical plan has been worked out and additional identification and sensitivities performed as required.

#### 4.7 Antimicrobial Susceptibility Testing

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

#### 4.8 Referral for Outbreak Investigations

N/A

#### 4.9 Referral to Reference Laboratories

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

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

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

England and Wales

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>

Scotland

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

Northern Ireland

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

## 5 Reporting Procedure

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### 5.1 Microscopy

N/A

### 5.2 Culture

Report all organisms.

Report absence of growth.

Also, report results of supplementary investigations

#### 5.2.1 Culture Reporting Time

Written report: 16hr–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.

### 5.3 Antimicrobial Susceptibility Testing

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

## 6 Notification to PHE<sup>51,52</sup> or Equivalent in the Devolved Administrations<sup>53-56</sup>

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

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

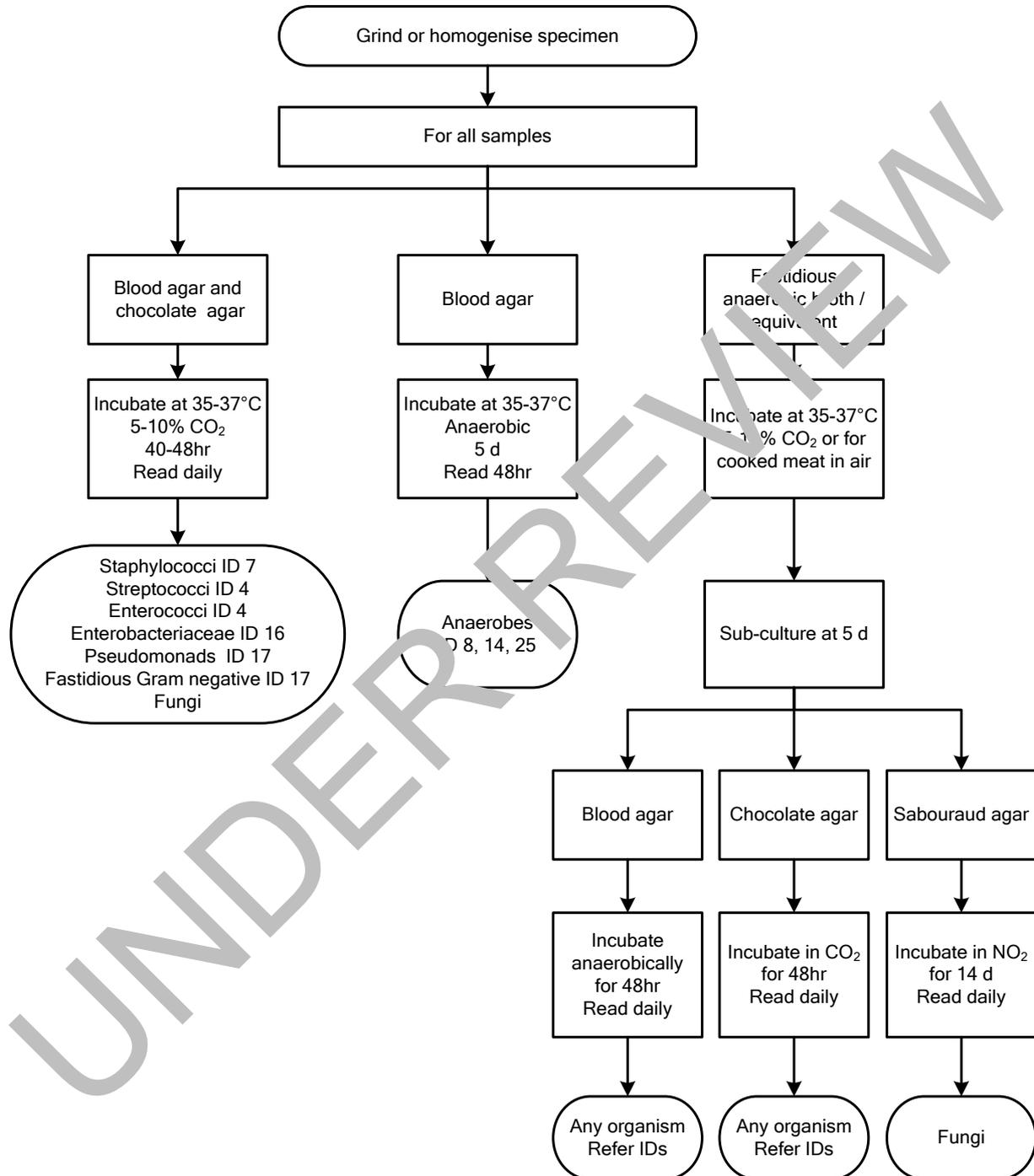
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local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

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

<http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/>

Other arrangements exist in [Scotland](#)<sup>53,54</sup>, [Wales](#)<sup>55</sup> and [Northern Ireland](#)<sup>56</sup>

## Appendix: Investigation of Prosthetic Joint Infection Samples



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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".

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