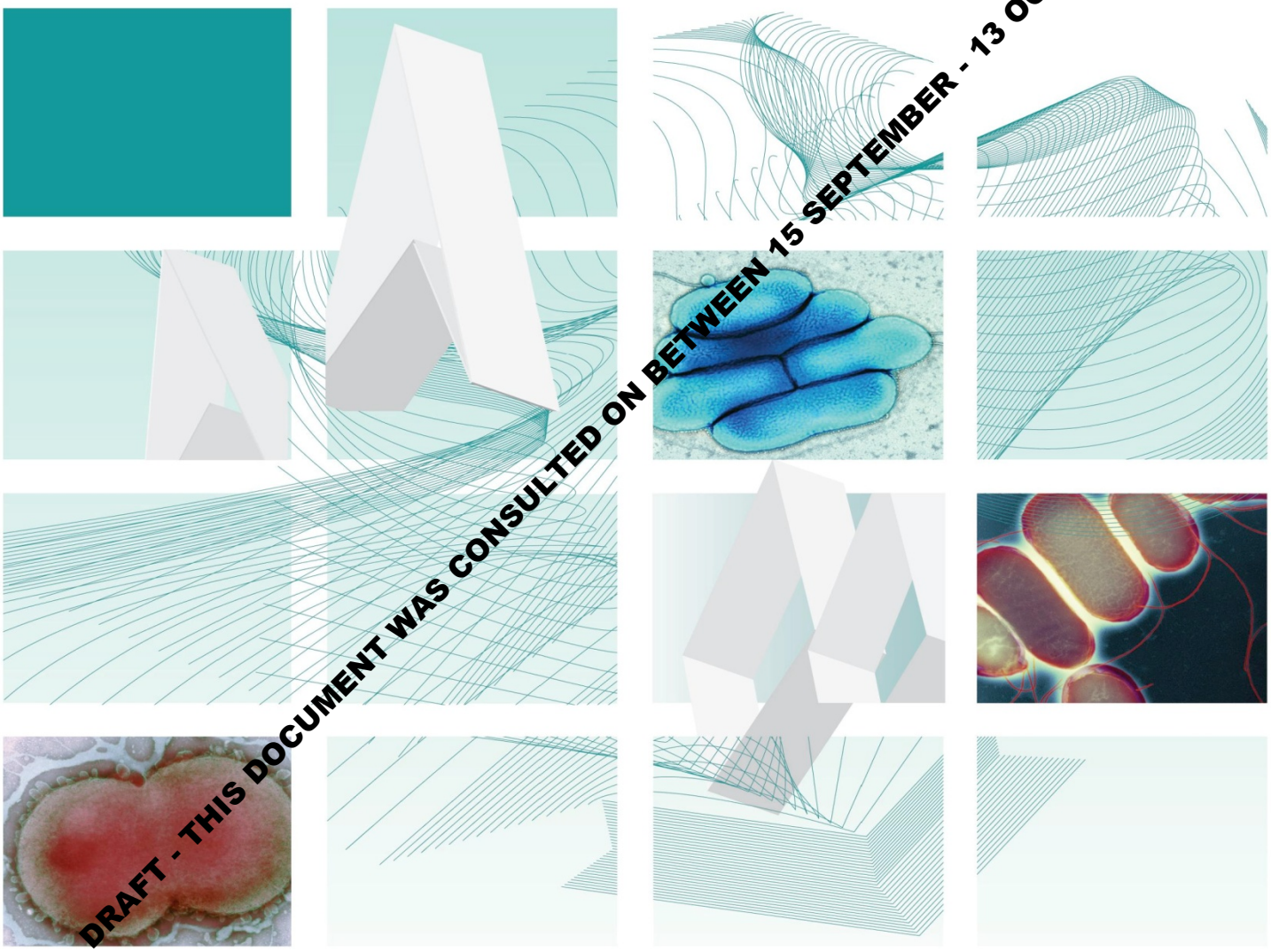
Public Health England

NHS

UK Standards for Microbiology Investigations

Investigation of Orthopaedic Implant Associated Infections



NICE accredited
www.nice.org.uk/accreditation

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 44 | Issue no: dv+ | Issue date: dd.mm.yy <tab+enter> | Page: 1 of 30

Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website 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).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

We also acknowledge Dr Bridget Atkins of the Bone Infection Unit, Nuffield Orthopaedic Centre, Oxford and the UK Standards for Microbiology Investigation Working Group for Clinical Bacteriology for their considerable specialist input.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: http://www.hpa.org.uk/SMI

UK Standards for Microbiology Investigations are produced in association with:

Contents

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

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

Amendment No/Date.	4/dd.mm.yy <tab+enter>
Issue no. discarded.	1.3
Insert Issue no.	
Section(s) involved	**Amendment**

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

UK SMI[#]: 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. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

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

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

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

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

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313.

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.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (YYYY <tab+enter>). Investigation of Orthopaedic Implant Associated Infections. UK Standards for Microbiology Investigations. B 44 Issue. http://www.hpa.org.uk/SMI/pdf

Scope of Document

Type of Specimen

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

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 arthopathies 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[1]. 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[2].

Risk Factors for Infection[3]

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[4,5]. The risk of infection in a joint replacement is increased by patient factors, including; the early development of a surgical site infection not apparently involving the prosthesis, a National Nosocominal Infections Surveillance Score of one or two, the presence of malignancy and previous joint arthroplasty[3]. 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[6].

Pathogenesis and Microbiology

Organisms may be introduced into the joint during primary implantation surgery or via a haematogenous (bloodstream) route[7]. 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[7]. 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[4,8,9].

Once infection is established around a prosthetic joint, organisms can form a 'biofilm'[10]. 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[11]. 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 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 raised[11]. 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[11]. 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[12]. 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 sample for testing 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%[13,14].

A semi-quantitative white cell count on the synovial fluid is useful for differentiating inflammatory from non-inflammatory arthritides; however it is less useful at differentiating infection from inflammation[2]. A total synovial cell count may be helpful in certain clinical situations[15-17]. Cut off values for synovial fluid leucocyte count and differential cell counts for the diagnosis of prosthetic joint infection have been determined in several studies. Leukocyte cut offs ranged from 1100 cells/µL to over 4000 cells/ µL[18-22]. Leukocyte differentials ranged from >64% to >80% neutrophils[18-22]. These cut off values are lower than those in cases of septic arthritis[18,19]. Specificity and sensitivity varied ranging from 82 to 98% and 84 to 97% respectively[18-22]. In patients with underlying inflammatory disease, counts may be high even in the absence of infection. When appropriate, synovial fluid should also be examined for crystals. A synovial biopsy may also be considered.

The approximate cut off for acute prosthetic joint infection, applicable to tests taken within six weeks of most recent surgery, as agreed (strong consensus) at the proceedings of the international consensus meeting on periprosthetic joint infections are as follows[23]:

- synovial white blood cell count (WBC) >10,000 cells/µL
- synovial neutrophil percentage (PMN%) >90%

The approximate cut off for chronic prosthetic joint infection, applicable to tests taken more than six weeks after the most recent surgery, are as follows[23]:

- synovial white blood count (WBC) >3,000 cells/µL
- synovial neutrophil percentage (PMN%) >80%

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.

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 surgical decision making[24,25].

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[26]

Samples can be transferred to the laboratory using routine timescales (ie within hours rather than minutes). There are no published comparisons or validations of various tissue processing methods in the orthopaedic setting. The method of shaking with glass beads is relatively simple and carries a low risk of contamination which has been shown experimentally to be superior to shaking in broth alone in the recovery of Bacillus spores from polymer surfaces[27]. 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[28].

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[1,29-31].

Microscopy and Culture[32]

Gram staining in elective revision cases should not be considered for diagnosing infection as it has extremely poor sensitivity[13,14,23,33,34].

Gram staining can however be used in acute infection to distinguish between aggregates of ultrasound-dislodged biofilm bacteria from other debris and contaminating bacteria[14]. These can appear as single cells or very small groups of cells. A negative Gram stain does not however rule out infection. False positive Gram stains associated with periprosthetic infections are rare[13].

Culture methods should include an enrichment broth. Cooked meat broth and continuous monitoring blood culture systems (CMBCS) have equivalent sensitivity, and are more sensitive than fastidious anaerobic agar plates in orthopaedic device related infection[35-38]. In chronic infections, primary plates may be omitted provided multiple samples are taken and enrichment broths used.

Where primary plates are used they should 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[39]. Thymidine dependent auxotrophs usually do not grow on blood agar and have atypical colonial appearance resembling haemophili or streptococci on chocolate agar[40]. The true prevalence and clinical relevance of small colony forms in prosthetic joint infection is unclear.

Automation[41]

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[35,36].

Duration of culture

Traditionally orthopaedic samples have been cultures for up to five days. More recently evidence suggested that incubation of up to 14 days may be necessary to isolate less virulent organisms such as propionibacteria and diphtheroids[23,42,43]. 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. Some broth enrichment methods require incubation for 14 days, however in a protocol based on vortexing with sterile beads and enrichment in cooked meat broth with terminal subculture after 5 days the sensitivity of the broth enrichment after 5 days was almost equivalent to the sensitivity obtained with two blood cultures (aerobic and anaerobic) despite the lower inoculum used for the broth. If a set of aerobic and anaerobic bottles are used for the enrichment an analysis of receiver-operator characteristics (ROC) has demonstrated that there is no need for incubation times exceeding 5 days[36,38]. Full automation using CMBCS bottles suggests that > 98% of significant results have flagged within 3 days[38].

This SMI therefore recommends up to 5 days culture using either cooked meat broth or continuous monitoring blood culture system methods[22,38,44]. However, in cases of suspected prosthetic joint infection, with low virulence organisms, or where preoperative cultures have failed to show growth and the clinical picture is consistent with prosthetic joint infection, culture may be extended to 14 days[23].

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[2]. 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[2]. 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[2]. 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[2]. 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[2].

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[45,46]. 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[47].

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[48].

Molecular methods

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[50]. 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[29,51]. 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[11,52].

MALDI-TOF Mass Spectrometry[53,54]

Recent developments in identification of bacteria, and fungi include the use of 16s ribosomal protein profiles obtained by Matrix Assisted Laser Desorption Ionisation –

Time of Flight (MALDI-TOF) mass spectroscopy[53]. 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[50].

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.

Specimen Containers[55,56]

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”.

Sonication containers are available. See below.

Sonication[26]

Gram positive bacteria have been found to be resistant to the effect of ultrasound; Gram negative organisms may be more susceptible[31]. 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.

Contamination

Repetitive subculture from the enrichments broth during incubation to may lead to contamination; use of continuous monitoring blood culture bottles flag when positive, thereby reducing the risk of contamination[36].

Effect of Antibiotic Use

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. 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)[57].

Revision arthroplasty 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.

1 Safety Considerations[55,56,58-72]

1.1 Specimen Collection, Transport and Storage[55,56,58-61]

Care should be taken to avoid accidental injury when using “sharps”. The use of sharp objects should be avoided wherever possible. Sterile, needleless syringes and blood transfer devices are commercially available which may be used for the aseptic transfer of sample homogenate into blood culture bottles.

Use aseptic technique.

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

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[55,56,58-72]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[64].

Ideally, microbiological analysis should be carried out in a Class II cabinet, using aseptic technique, to reduce the risk of contamination of the sample and to protect the user[32,36].

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

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[73]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[73].

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

Swabs are to be discouraged. However if sent, swabs for bacterial and fungal culture should be placed into appropriate transport medium and transport in sealed plastic bags.

2.3 Adequate Quantity and Appropriate Number of Specimens[73]

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 1mL.

Small volumes of synovial fluid (<1mL) may impede the recovery of organisms.

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

3 Specimen Transport and Storage[55,56]

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[73]

If processing is delayed, refrigeration is preferable to storage at ambient temperature[73].

4 Specimen Processing/Procedure[55,56]

4.1 Test Selection

N/A

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment[27,28]

Soft tissue samples

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 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 glass beads. Glass beads and Ringer's or saline can be added in the laboratory, maintaining asepsis diligently. Ideally processing of samples should take place in a Class II cabinet[32,36]. Homogenisation with glass beads can be performed, for example, by shaking at 250 rpm for 10 minutes in a covered rack on an orbital shaker or, alternatively, vortexing for 15 seconds (40Hz) (alternative methods of homogenisation may also be used)[25].

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 2mL 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.

4.3.2 Specimen processing

If available, microbiological analysis should be carried out in a Class II cabinet, using aseptic technique, to reduce the risk of contamination of the sample and to protect the user[32,36].

Inoculate plates and broth after homogenisation of soft tissue samples and prosthesis, or directly from aspirate fluid. 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 (see B 40 - Investigation of Specimens for *Mycobacterium* species). 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 5 days, examining daily for evidence of growth. Subculture if cloudy, but otherwise perform a terminal subculture at 5 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 5 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[39,74]. 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.

4.4 Microscopy (refer TP 39 – Staining Procedures)

4.4.1 Standard

N/A

4.4.2 Supplementary

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 may 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.

Total white cell counts and differential leucocyte counts may be performed on joint aspirates.

Total white cell count

The presence of a clot will invalidate a cell count.

Perform a total cell count on the synovial fluid in a counting chamber.

Differential leucocyte count - Toluidine blue/Methylene blue stain (Wright stain) (refer to TP 39 - Staining Procedures)

Differentiating between polymorphonuclear leucocytes and mononuclear leucocytes may be performed in two ways:

- **Counting chamber method: recommended for lower WBC counts.**

  Note: When a particle counter has been validated for use with cells in this context then it may be used[75].

  a) Non- or lightly-bloodstained specimens

  - Stain the fluid with 0.1% stain solution such as toluidine, methylene or nile blue. This stains the leucocyte nuclei thus aiding differentiation of the cells
  - The dilution factor must be considered when calculating the final cell count
  - Count and record the numbers of each leucocyte type
  - Express the leucocyte count as number of cells per litre

  b) Heavily bloodstained specimens

  - Dilute specimen with WBC diluting fluid and leave for 5 minutes before loading the counting chamber. This will lyse the red blood cells and stain the leucocyte nuclei for differentiation
  - Count and record the number of each leucocyte type: the dilution factor must be considered when calculating the final cell count
  - Express the leucocyte count as number of cells per litre

- **Stained method**

Recommended for very high WBC counts where differentiation in the counting chamber is difficult

- Prepare a slide from the centrifuged deposit or cytospin preparations as for the Gram stain but allow to air dry
- Fix in alcohol and stain with a stain suitable for WBC morphology

  Note: Heat fixation distorts cellular morphology
- Count and record the number of each leucocyte type as a percentage of the total

4.5 Culture and Investigation

Inoculate each agar plate with sample using a sterile pipette (Q 5 - Inoculation of Culture Media for Bacteriology).

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

4.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	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)	All specimens	Blood agar and Chocolate agar	35 - 37	5-10% CO_2	40-48hr	Daily	Staphylococci Streptococci Enterococci Enterobacteriaceae Fastidious Gram negatives Pseudomonads Yeast Mould
		FAA*	35 - 37	Anaerobic	5 d	3 d and 5 d	Anaerobes
		Fastidious anaerobic, cooked meat broth or equivalent. **Subculture if evidence of growth, or at day 5 on plates as below: or ***blood culture for CMBCS. (aerobic and anaerobic bottles[37]) Subculture when flags positive on plates as below:	35 - 37	Air	5d **** or up to 5 d	Daily	Any
		Blood agar	35-37	Anaerobic	40-48hr	Daily	Any Anaerobes
		Chocolate agar	35 - 37	5-10% CO_2	40-48hr	Daily	Any

Clinical details/ conditions	Specimen	Supplimentary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Fungal infection suspected	All specimens	Sabouraud agar	28-30	Air	14 d	Daily	Yeast and Mould
Always consider other organisms such as *Mycobacterium* species (B 40 - Investigation of Specimens for *Mycobacterium* species), fungi and actinomycetes. *Neomycin FAA with metronidazole 5µg disc may be used dependent on local policy. **Subcultures should be examined periodically (ideally daily) and subcultured if there is evidence suggestive of growth. Terminal subcultures should be performed at 5 days. *** Blood culture subcultures should be performed when the bottle flags positive. A terminal subculture at 5 days is not required[38,44]. **** Incubation may be extended if clinically indicated. For example in cases of suspected prosthetic joint infection with low virulence organisms, or where preoperative cultures have failed to show growth, and the clinical picture is consistent with prosthetic joint infection, culture may be extended to up to 14 days.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Actinomycetes	genus level ID 15 – Identification of Anaerobic *Actinomyces* species
Anaerobes	genus level ID 14 - Identification of Anaerobic Cocci ID 8 - Identification of *Clostridium* species ID 25 - Identification of Anaerobic Gram negative rods
β-haemolytic streptococci	Lancefield group level or species level
Other streptococci	species level
Enterococci	species level
Enterobacteriaceae	species level
Yeast and Mould	species level
Haemophilus species	species level
Pseudomonads	species level
S. aureus	species level
Staphylococci (not aureus)	to coagulase negative Staphylococci or species level (if multiple samples)
Mycobacterium species	B 40 - Investigation of Specimens for *Mycobacterium* species

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

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.

4.7 Antimicrobial Susceptibility Testing

Extensive antibiograms (including rifampicin) are required[16,38,44]. It is important to include a wide range of antibiotics particularly for those patients who may require prolonged oral treatment with biofilm active drugs. These antibiotics are not usually included in the common first line antimicrobials tested in most laboratories. For Gram positive organisms these may include a teicoplanin MIC plus antibiotics such as rifampicin, tetracycline, quinolone, co-trimoxazole, fusidic acid, linezolid, and others.

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

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

Where clinically or epidemiologically indicated, or following detection of organisms with unusual or unexpected resistance, or whenever there is a laboratory or clinical problem, or anomaly that requires elucidation, isolates 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

5.1 Microscopy

5.1.1 Standard

N/A

5.1.2 Supplementary

Gram stain

Report on organisms detected.

Note: Not to be used for diagnosis of chronic prosthetic joint infection.

White cell count

Report numbers of WBCs x 10^6 per litre.

Differential count

Report mononuclear leucocytes as percentage of the total WBCs

5.1.3 Thresholds for white blood cell count and neutrophil percentage

	Approximate cut off for acute PJI ≤ 6 week after most recent surgery	Approximate cut off for acute PJI > 6 week after most recent surgery
Synovial white blood cell count (WBC)	>10,000 cells/µL	>3,000 cells/µL
Synovial neutrophil percentage (PMN%)	>90%	>70%

5.2 Culture

Report all organisms.

or

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.

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[76,77] or Equivalent in the Devolved Administrations[78-81]

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.

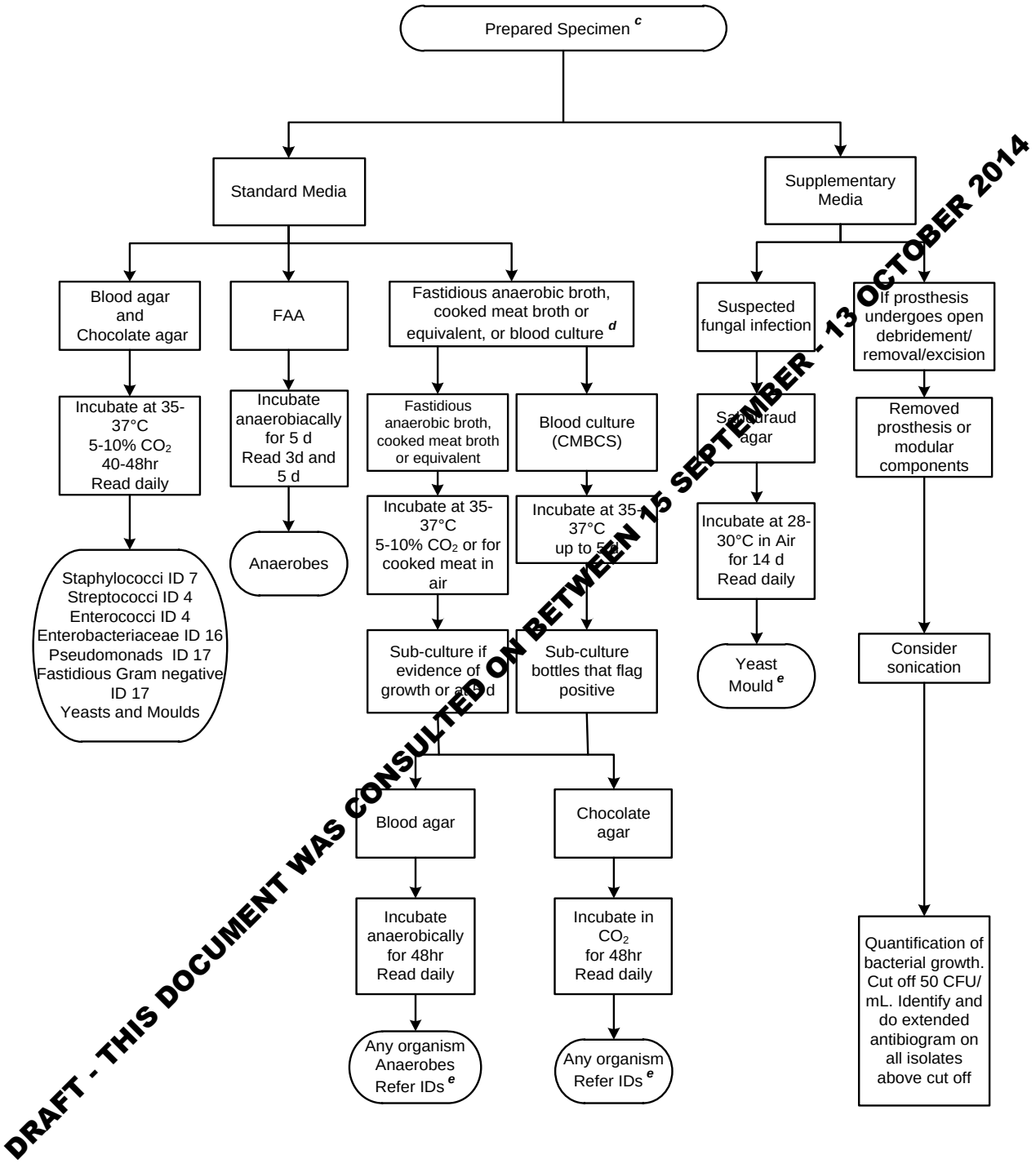
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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[78,79], Wales[80] and Northern Ireland[81].

Appendix 1: Culture [a, b]



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) Microbiological tests may not be required for synovial biopsy specimens if proceeding straight to revision/removal.

c) 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).

d) 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.

e) Interpretation of intra-operative samples: Two or more samples with an indistinguishable organism are a positive microbiology result.

References

1. Vergidis P, Greenwood-Quaintance KE, Sanchez-Sotelo J, Morrey BF, Steinmann SP, Karau MJ, et al. Implant sonication for the diagnosis of prosthetic elbow infection. J Shoulder Elbow Surg 2011;20:1275-81.

2. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, et al. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. J Clin Microbiol 1998;36:2932-9.

3. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, et al. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998;27:1247-54.

4. Moran E, Masters S, Berendt AR, McLardy-Smith P, Byren I, Atkins BL. Guiding empirical antibiotic therapy in orthopaedics: The microbiology of prosthetic joint infection managed by debridement, irrigation and prosthesis retention. J Infect 2007;55:1-7.

5. Societe de Pathologie infectious de Langue Francaise. Recommendations for bone and joint prosthetic device infections in clinical practice (prosthesis, implants, osteosynthesis). Societe de Pathologie Infectieuse de Langue Francaise. Med Mal Infect 2010;40:205-211.

6. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, et al. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-15.

7. Murdoch DR, Roberts SA, Fowler JV, Jr., Shah MA, Taylor SL, Morris AJ, et al. Infection of orthopedic prostheses after Staphylococcus aureus bacteremia. Clin Infect Dis 2001;32:647-9.

8. Marculescu CE, Berbari EF, Cockerill FR, III, Osmon DR. Unusual aerobic and anaerobic bacteria associated with prosthetic joint infections. Clin Orthop Relat Res 2006;451:55-63.

9. Marculescu CE, Berbari EF, Cockerill FR, III, Osmon DR. Fungi, mycobacteria, zoonotic and other organisms in prosthetic joint infection. Clin Orthop Relat Res 2006;451:64-72.

10. Gristina AG, Naylor P, Myrvik Q. Infections from biomaterials and implants: a race for the surface. Med Prog Technol 1988;14:205-24.

11. Esposito S, Leone S. Prosthetic joint infections: microbiology, diagnosis, management and prevention. Int J Antimicrob Agents 2008;32:287-93.

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

13. Oethinger M, Warner DK, Schindler SA, Kobayashi H, Bauer TW. Diagnosing periprosthetic infection: false-positive intraoperative Gram stains. Clin Orthop Relat Res 2011;469:954-60.

14. Johnson AJ, Zywiel MG, Stroh DA, Marker DR, Mont MA. Should gram stains have a role in diagnosing hip arthroplasty infections? Clin Orthop Relat Res 2010;468:2387-91.

15. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med 2004;117:556-62.

16. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of america. Clin Infect Dis 2013;56:e1-e25.

17. Ghanem E, Parvizi J, Burnett RS, Sharkey PF, Keshavarzi N, Aggarwal A, et al. Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. J Bone Joint Surg Am 2008;90:1637-43.

18. Gomez E, Patel R. Laboratory Diagnosis of Prosthetic Joint Infection, Part I. Clinical Microbiology Newsletter 2011;33:55-60.

19. Dinneen A, Guyot A, Clements J, Bradley N. Synovial fluid white cell and differential count in the diagnosis or exclusion of prosthetic joint infection. Bone Joint J 2013;95-B:554-7.

20. Parvizi J, Ghanem E, Sharkey P, Aggarwal A, Burnett RS, Barrack RL. Diagnosis of infected total knee: findings of a multicenter database. Clin Orthop Relat Res 2008;466:2628-33.

21. Ghanem E, Parvizi J, Burnett RS, Sharkey PF, Keshavarzi N, Aggarwal A, et al. Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. J Bone Joint Surg Am 2008;90:1637-43.

22. Schinsky MF, Della Valle CJ, Sporer SM, Paprosky WG. Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. J Bone Joint Surg Am 2008;90:1869-75.

23. Parvizi J, Gehrke T, Chen AF. Proceedings of the International Consensus on Periprosthetic Joint Infection. Bone Joint J 2013;95-B:1450-2.

24. Athanasou NA, Pandey R, de Steiger R, Crook D, Smith PM. Diagnosis of infection by frozen section during revision arthroplasty. J Bone Joint Surg Br 1995;77:28-33.

25. Tsaras G, Maduka-Ezeh A, Inwards CY, Mabry T, Erwin PJ, Murad MH, et al. Utility of intraoperative frozen section histopathology in the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. J Bone Joint Surg Am 2012;94:1700-11.

26. Del AA, Bertrand ML. The diagnosis of periprosthetic infection. Open Orthop J 2013;7:178-83.

27. 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.

28. Roux AL, Sivadon-Tardy V, Bauer T, Lortat-Jacob A, Herrmann JL, Gaillard JL, et al. Diagnosis of prosthetic joint infection by beadmill processing of a periprosthetic specimen. Clin Microbiol Infect 2011;17:447-50.

29. Esteban J, Alonso-Rodriguez N, del-Prado G, Ortiz-Perez A, Molina-Manso D, Cordero-Ampuero J, et al. PCR-hybridization after sonication improves diagnosis of implant-related infection. Acta Orthop 2010;83:299-304.

30. Holinka J, Bauer L, Hirschl AM, Graninger W, Windhager R, Presterl E. Sonication cultures of explanted components as an add-on test to routinely conducted microbiological diagnostics improve pathogen detection. J Orthop Res 2011;29:617-22.

31. Monsen T, Lovgren E, Widerstrom M, Wallinder L. In vitro effect of ultrasound on bacteria and suggested protocol for sonication and diagnosis of prosthetic infections. J Clin Microbiol 2009;47:2496-501.

32. Velay A, Schramm F, Gaudias J, Jaulhac B, Riegel P. Culture with BACTEC Peds Plus bottle compared with conventional media for the detection of bacteria in tissue samples from orthopedic surgery. Diagn Microbiol Infect Dis 2010;68:83-5.

33. Ghanem E, Ketonis C, Restrepo C, Joshi A, Barrack R, Parvizi J. Periprosthetic infection: where do we stand with regard to Gram stain? Acta Orthop 2009;80:37-40.

34. Spangehl MJ, Masterson E, Masri BA, O'Connell JX, Duncan CP. The role of intraoperative gram stain in the diagnosis of infection during revision total hip arthroplasty. J Arthroplasty 1999;14:952-6.

35. Font-Vizcarra L, Garcia S, Martinez-Pastor JC, Sierra JM, Soriano A. Blood culture flasks for culturing synovial fluid in prosthetic joint infections. Clin Orthop Relat Res 2010;468:2238-43.

36. Hughes HC, Newnham R, Athanasou N, Atkins BL, Bejon P, Bowler IC. Microbiological diagnosis of prosthetic joint infections: a prospective evaluation of four bacterial culture media in the routine laboratory. Clin Microbiol Infect 2011;17:1528-30.

37. Jordan RW, Smith NA, Saithna A, Sprowson AP, Foguet P. Sensitivities, specificities, and predictive values of microbiological culture techniques for the diagnosis of prosthetic joint infection. Biomed Res Int 2014;2014:180416.

38. Minassian AM, Newnham R, Kalimeris E, Bejon P, Atkins BL, Bowler IC. Use of an automated blood culture system (BD BACTEC) for diagnosis of prosthetic joint infections: easy and fast. BMC Infect Dis 2014;14:233.

39. Looney WJ. Small-colony variants of Staphylococcus aureus. Br J Biomed Sci 2000;57:317-22.

40. Gilligan PH, Gage PA, Welch DF, Muszynski MJ, Wait KR. Prevalence of thymidine-dependent Staphylococcus aureus in patients with cystic fibrosis. J Clin Microbiol 1987;25:1258-61.

41. Minassian A, Newnham R, Kalimeris E, Bejon P, Atkins B, and Bowler I. BACTEC for diagnosis of prosthetic joint infections: easy, cheap and fast. IDSA IDWeek. San Francisco. 2013.

42. Schafer P, Fink B, Sandow D, Margull A, Berger I, Frommelt L. Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin Infect Dis 2008;47:1403-9.

43. Larsen LH, Lange J, Xu Y, Schonheyder HC. Optimizing culture methods for diagnosis of prosthetic joint infections: a summary of modifications and improvements reported since 1995. J Med Microbiol 2012;61:309-16.

44. Portillo MaE, Salvad+¦ M, Alier A, Mart+¡nez S, Sorli L, Horcajada JP, et al. Advantages of sonication fluid culture for the diagnosis of prosthetic joint infection. Journal of Infection 2014;69:35-41.

45. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007;357:654-63.

46. Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelo J, Osmon DR, et al. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. J Clin Microbiol 2009;47:1878-84.

47. Sousa L, Puig L, Torres-Claramunt R, Gonzalez A, Alier A, Knobel H, et al. The relationship between microbiology results in the second of a two-stage exchange procedure using cement spacers and the outcome after revision total joint replacement for infection: the use of sonication to aid bacteriological analysis. J Bone Joint Surg Br 2012;94:249-53.

48. Lambert PA, Van Maurik A, Parvatham S, Akhtar Z, Fraise AP, Krikler SJ. Potential of exocellular carbohydrate antigens of Staphylococcus epidermidis in the serodiagnosis of orthopaedic prosthetic infection. J Med Microbiol 1996;44:355-61.

49. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev 2006;19:165-256.

50. Arciola CR, Montanaro L, Costerton JW. New trends in diagnosis and control strategies for implant infections. Int J Artif Organs 2011;34:727-36.

51. Cherkaoui A, Ceroni D, Emonet S, Lefevre Y, Schrenzel J. Molecular diagnosis of Kingella kingae osteoarticular infections by specific real-time PCR assay. J Med Microbiol 2009;58:65-8.

52. Bjerkan G, Witso E, Nor A, Viset T, Loseth K, Lydersen S, et al. A comprehensive microbiological evaluation of fifty-four patients undergoing revision surgery due to prosthetic joint loosening. J Med Microbiol 2012;61:572-81.

53. Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti JL, et al. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clin Biochem 2011;44:104-9.

54. van Veen SQ, Claas ECJ, Kuijper EJ. High-Throughput Identification of Bacteria and Yeast by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry in Conventional Medical Microbiology Laboratories â–¿. J Clin Microbiol 2010;48:900-7.

55. European Parliament. UK Standards for Microbiology Investigations (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".

56. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.

57. Geipel U. Pathogenic organisms in hip joint infections. Int J Med Sci 2009;6:234-40.

58. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.

59. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.

60. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.

61. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).

62. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32

63. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.

64. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.

65. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.

66. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.

67. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.

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

69. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

70. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.

71. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.

72. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14

73. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, Jr., et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis 2013;57:e22-e121.

74. Maduka-Ezeh AN, Greenwood-Quaintance KE, Karau MJ, Berbari EF, Osmon DR, Hanssen AD, et al. Antimicrobial susceptibility and biofilm formation of Staphylococcus epidermidis small colony variants associated with prosthetic joint infection. Diagn Microbiol Infect Dis 2012;74:224-9.

75. Penders J, Fiers T, Dhondt AM, Claeys G, Delanghe JR. Automated flow cytometry analysis of peritoneal dialysis fluid. Nephrol Dial Transplant 2004;19:463-8.

76. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37.

77. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.

78. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).

79. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.

80. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.

81. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).