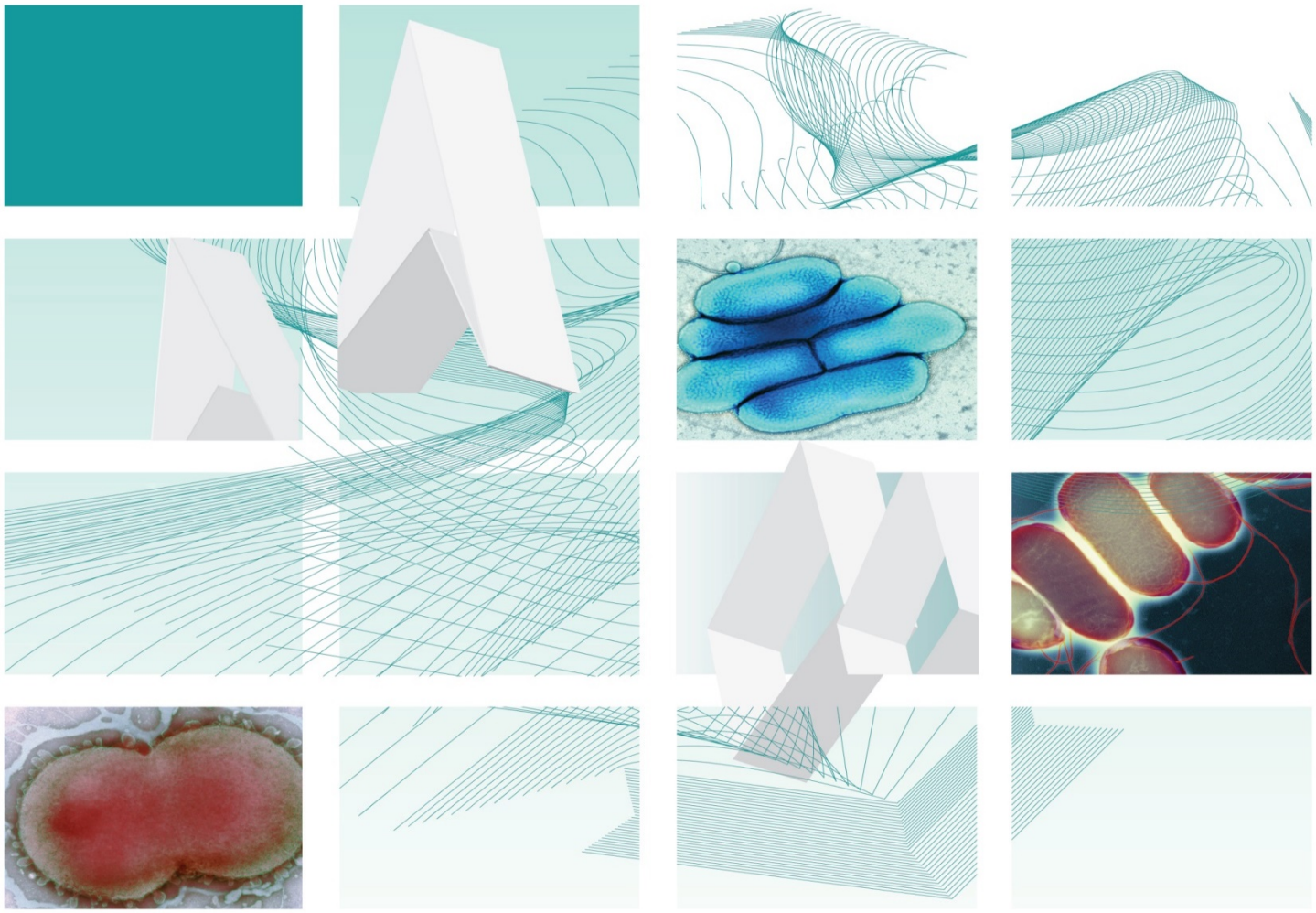
Public Health England

UK Standards for Microbiology Investigations

Investigation of orthopaedic implant associated infections



NICE accredited
www.nice.org.uk/accreditation

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

This publication was created by Public Health England (PHE) in partnership with the NHS.

Issued by the Standards Unit, National Infection Service, PHE

Bacteriology | B 44 | Issue number: 2.1 | Issue date: 18 August 21 | Page: 1 of 34

Acknowledgments

UK Standards for Microbiology Investigations (UK 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 UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see the Steering Committee page on GOV.UK).

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.

PHE publications gateway number: GOV-9410

UK Standards for Microbiology Investigations are produced in association with:

Contents

Amendment table

Each UK 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 number / date	5 / 18 August 2021
Issue number discarded	2
Insert issue number	2.1
Section(s) involved	Amendment
Whole document	Updated to new template
Appendix	Incubation of FA broth or equivalent corrected to 35-37 in air. Removed incubation at 5-10% CO2

Amendment number / date	4 / 23 February 2016
Issue number discarded	1.3
Insert issue number	2
Section(s) involved	Amendment
Whole document	Title changed to ‘Investigation of orthopaedic implant associated infections’. References updated throughout.
Scope	Fixation devices added to types of specimen. Scope updated to reflect new title and use of molecular techniques.
Introduction	Re-structured and streamlined for clarity. Information regarding sample types, sampling, sample processing and molecular methods inserted and updated (previously in Technical Information/Limitations). Synovial white cell count cut offs for acute and chronic prosthetic joint infection included.

	Section on rapid techniques expanded to include MALDI-TOF MS. Information on sonication included. Gram staining in elective revision cases should not be considered for diagnosing infection. Recommends up to 5 days culture using either cooked meat broth or continuous monitoring blood culture system method, extended to 14 days in some cases.
Technical information and limitations	Information included on sonication, small colony variants, contamination and effect of antibiotic use.
Safety considerations	Avoid sharps where possible. Needless syringes are available for use with blood culture bottles. Ideally analysis should be carried out in a Class II cabinet.
Specimen collection	If possible stop antibiotics 2 weeks prior to surgery. Swabs are discouraged. In theatres multiple (3 to 5) samples should be taken.
Specimen transport and storage.	IDSA recommend samples are processed within 2 hrs.
Specimen processing or procedure	Ideally analysis should be carried out in a Class II cabinet. Primary plates may not be required in some situations. Enrichment broth/blood culture broth should be incubated for 5 days (extended to 14 days in certain situations). Method for total white and differential leucocyte counts included. Culture media, conditions and organisms table updated: • Standard media o FAA, read at 3 and 5 day. o Inclusion of continuous monitoring blood culture system.

	o Broth incubation 5 days, extended to 14 days in certain situations. • Supplementary media o Sabouraud agar, incubate for 14 days. • Addition of molecular techniques (NAAT). Identification – isolates should be kept for at least 2 weeks. Antimicrobial susceptibility testing updated to include extensive antibiograms.
Reporting procedure	Updated to include total white and differential cell counts. Culture reporting updated in line with new template. Molecular reporting added.
Appendix	Updated in line with section 4.5.3 Culture media, conditions and organisms.

*Reviews can be extended up to 5 years subject to resources available.

1. General information

View general information related to UK SMIs.

2. Scientific information

View scientific information related to UK SMIs.

3. Scope of document

This UK SMI describes the microbiological investigation of orthopaedic implant associated infection samples and includes information on molecular techniques.

For information regarding bone and tissue samples associated with osteomyelitis refer to B 42 – Investigation of bone and soft tissue associated with osteomyelitis.

This UK SMI should be used in conjunction with other UK SMIs.

4. Background

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).

4.1 Risk factors for infection

Infection rates are now much lower than when joint replacement was first introduced. There is however still a risk associated with each procedure. This is around 1 to 2% for elective hip and knee replacements and higher for emergency trauma operations, for example, hemiarthroplasties (3, 4). 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 Healthcare Safety Network (NHSN) Score of 1 or 2, the presence of malignancy and previous joint arthroplasty(5 to 7). The NHSN score encompasses both surgical and patient factors such as the duration of surgery, degree of wound contamination and patient’s preoperative health (7). 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 (5, 8, 9).

Surveillance of surgical site infections may be used as a means to prevent such infections which are associated with considerable morbidity and extended hospital stay (10, 11).

4.2 Pathogenesis and microbiology

Organisms may be introduced into the joint during primary implantation surgery, via a haematogenous (bloodstream) route or from post-operative contamination of the wound(12). 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* bacteraemia may be associated with septic arthritis in those with pre-existing prosthetic joints (12). 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 (3, 13, 14).

Once infection is established around a prosthetic joint, organisms can form a 'biofilm'(15). 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 (16). 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 organisms which form biofilms should be used, but standard antimicrobial sensitivities may not predict the required antimicrobial activity (17). In vitro models testing activity of antimicrobials against organisms within a biofilm are not at present feasible in routine laboratories (9).

4.3 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(17). 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 not specific (17). These presentations are often difficult to differentiate from those of mechanical pain or aseptic loosening (18). The presence of a discharging sinus however, indicates the presence of a deep prosthetic joint infection.

4.4 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 early loosening, fracture or other pathology. Plain X-rays may show loosening, but do not differentiate septic from aseptic loosening. 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. 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 (19). A joint aspirate for cell count, culture and histology, 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.

4.5 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 to 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 1 or 2 samples are taken. When 5 samples are taken, the false positive rate with 2 or 3 samples positive is less than 5% whereas false positive rates close to 30% are seen with a single positive sample (2). Growth of an indistinguishable organism from 2 or more samples is 71% sensitive and 97% specific. Recovery of an indistinguishable organism from 3 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).

5. Safety considerations

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.

Compliance with postal, transport and storage regulations is essential.

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet (20).

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 (21, 22).

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

The above guidance should be supplemented with local COSHH and risk assessments (23 to 28).

6. Diagnostic tests and investigation

6.1 Culture and microscopy

6.1.1 Specimen type

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

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 less than 10% (29, 30).

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 (31 to 33). 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 (34, 35). Leukocyte cut offs ranged from 1100 cells/µL to over 4000 cells/µL (33, 36 to 39). Leukocyte differentials ranged from 64% to greater than or equal to 80% neutrophils (33, 36 to 39). These cut off values are lower than those in cases of septic arthritis (36, 37). Specificity and sensitivity varied ranging from 82 to 98% and 84 to 97% respectively (33, 36 to 39). 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 cut offs for acute prosthetic joint infection occurring within 6 weeks of surgery, as agreed (strong consensus) at the proceedings of the international consensus meeting on periprosthetic joint infections, are as follows (35):

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

The approximate cut off for chronic prosthetic joint infection, applicable to tests taken more than 6 weeks after the most recent surgery, are as follows (35):

- synovial white blood count (WBC) greater than 3,000 cells/µL
- synovial neutrophil percentage (PMN%) greater than 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 carried out if multiple biopsies can be performed.

Intra-operative biopsy

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 (40, 41).

Explanted prostheses

Explanted prostheses can be sent for microbiological investigation. They are often difficult to handle unless especially large pots are used 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.

Sonication, when used as an addition to conventional culture has been shown to improve the sensitivity of prosthetic joint infection microbiological diagnosis (42 to 44). It uses ultrasound to disrupt the bacterial biofilm on the prosthetic material. The improvement in sensitivity is most markedly seen in patients on antibiotics within 14 days prior to surgery (42, 45).

6.1.2 Pre-laboratory processes (pre analytical stage)

This section covers specific safety considerations related to this UK SMI (23, 24) (46), and should be read in conjunction with the generic safety considerations on GOV.UK.

Specimen collection, transport and storage:

For safety considerations refer to Section 2.

Collect specimens before antimicrobial therapy where possible (46).

If possible stop all antibiotics at 2 weeks prior to sampling and consider not giving routine surgical prophylaxis until after sampling (42, 47, 48).

To enable timely clinical management, samples should be processed urgently.

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.

For aspirates and radiologically guided biopsies, it is usually only possible to send one sample to microbiology. In theatres, multiple (3 to 5) 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 (less than 1mL) may impede the recovery of organisms.

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

Specimens should be transported and processed as soon as possible (46).

The Infectious Diseases Society of America (IDSA) guidelines recommend that specimens should be transported at room temperature, and should be processed immediately, and within a maximum of 2hr (46).

If processing is delayed, refrigeration is preferable to storage at ambient temperature (46).

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 1 and 2 stage exchange.

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

Pre-treatment

Soft tissue samples (49, 50)

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 (21, 22). 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) (41).

The diluent for the glass beads and tissue 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.

6.1.3 Laboratory processes (analytical stage)

Specimen processing

Samples can be transferred to the laboratory using routine timescales (that is, 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 (50). 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 (49).

Sonication of removed components has been examined as a means of disrupting bacterial biofilm in vascular and orthopaedic prostheses(51). A considerable number of studies have now been performed comparing sonication of the prosthesis in a sterile pot to conventional cultures (18, 51 to 55). Several centres have now adopted this as routine practice (1, 56 to 58).

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 (21, 22).

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). Primary plates may not be required in elective revisions, in high volume units and skilled multiple site sampling.

In addition, place some of the solution into an enrichment broth. If mycobacterial cultures are required this solution can then be used to inoculate mycobacterial cultures. This is best done 24 hours after the primary plates have been examined once, to decide if decontamination of the sample is required. For information regarding incubation conditions and duration refer to B 40 – Investigation of specimens for *Mycobacterium* species.

Incubate the enrichment broth for 5 days (culture may be extended to 14 days in certain situations), examining daily for evidence of growth (35). 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 (extended to 14 days in certain situations) (21, 35, 59 to 61). 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 under magnification for small-colony variants (62, 63). Care should be taken to distinguish small tissue fragments on the plate from small colonies. Small colony variants are often thymidine-dependent, 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.

Optional

Specimens collected into appropriate CE marked leak proof containers should be used for microscopy and may be used for molecular techniques. Specimens for molecular testing should be processed according to manufacturer's instructions (23, 24).

6.2 Microscopy and culture

Microscopy

Gram staining in elective revision cases should not be considered for diagnosing infection as it has extremely poor sensitivity (29, 30, 35, 64, 65).

Gram staining can however be used in acute infection to distinguish between aggregates of ultrasound-dislodged biofilm bacteria from other debris and contaminating bacteria (30). 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, but may have severe consequences if used as the basis for treatment (29). One study investigating false positive intraoperative Gram stains in diagnosing periprosthetic infection reported a sensitivity and specificity of 9% and 99% respectively (29). Another study concluded that Gram stains have a limited application, and reported a sensitivity and specificity of 7% and 92% respectively (66).

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 (21, 60, 67, 68).

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 and differential leucocyte count

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)

Note: When a particle counter has been validated for use with cells in this context then it may be used (69).

Differentiating between polymorphonuclear leucocytes and mononuclear leucocytes may be performed in 2 ways:

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

  a) Non- or lightly-blood stained 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

6.2.1 Laboratory processes (analytical stage)

Culture

Sample preparation

For safety considerations refer to Section 2.

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.

Duration of culture

Traditionally orthopaedic samples have been cultured for up to 5 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 (35, 70 to 74). The methods described omit any early subculture unless broths are cloudy. Visual inspection of broth media is not very accurate; many earlier positives may have been missed and time to detection may depend on how frequently cultures are examined (75). 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 2 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 (21, 60). Full automation using CMBCS bottles suggests that greater than 98% of significant results have flagged within 3 days (60).

This UK SMI therefore recommends up to 5 days culture using either cooked meat broth or continuous monitoring blood culture system methods (51, 60, 61). 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 (35, 59, 76).

Specimen processing

Table 1: Culture media, conditions and organisms

Clinical details and/or 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 to 37	5 to 10% CO_2	40 to 48hr	Daily	Staphylococci Streptococci Enterococci Enterobacteriaceae Fastidious Gram negatives Pseudomonads Yeast Mould
		FAA	35 to 37	Anaerobic	5d	3d and 5d	Anaerobes

Clinical details and/or conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
		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(68)) Subculture when flags positive on plates as below:	35 to 37	Air	5d *** or up to 5d ***	Daily	Any
		Blood agar	35 to 37	Anaerobic	40 to 48hr	Daily	Any anaerobes
		Chocolate agar	35 to 37	5 to 10% CO_2	40 to 48hr	Daily	Any
Fungal infection suspected(77)	All specimens	Sabouraud agar (slopes)	28 to 30	Air	14d	Daily	Yeast and mould

Clinical details and/or conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Optional molecular techniques							
Clinical details and/or conditions	**Specimen**	**Molecular technique**	**Instructions**				**Target organism(s)**
All clinical conditions	Bone marrow	NAAT	Follow manufacturer's instructions				Any organism

Always consider other organisms such as *Mycobacterium* species (B 40 – Investigation of specimens for *Mycobacterium* species), fungi and actinomycetes.

* Broths 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 (51, 60).

*** 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.

Identification

Refer to individual UK SMIs for organism identification.

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.

Automation(60)

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 (21, 67).

6.3 Rapid techniques

6.3.1 Serology

Serological techniques used for diagnosis of prosthetic joint infection have been studied in the research setting but have not yet been found to be of practical clinical benefit. The problem tends to be with specificity (78).

6.3.2 Molecular methods

Nucleic acid amplification techniques (NAAT) (36, 79 to 86)

NAAT – Nucleic acid amplification techniques (for example, PCR) for the identification of bacteria, fungi, parasites and viruses from clinical samples have been shown to be highly specific and sensitive. PCR targets conserved genes of the genome, and enables the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short timeframe particularly if multiplex real-time PCR is used.

NAAT 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 (79 to 82). PCR has been shown to be more sensitive than convention culture for the isolation of some fastidious organisms for example *Kingella kingae*, and PCR–hybridization after sonication has been shown to improve diagnosis of implant related infections (56, 87). There are however some issues with NAAT 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 tissue samples, and antibiotic susceptibility information is not available (17, 88).

Future developments may include the coupling of broad range PCR with next generation sequencing allowing analysis of the total microbiome associated with orthopaedic implant associated infections (83).

6.3.3 MALDI-TOF mass spectrometry

Recent developments in identification of bacteria, and fungi include the use of protein profiles obtained by Matrix assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectrometry (89). 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 (79).

Limited data is available on the use of MALDI-TOF MS in orthopaedic implant associated infection diagnosis; however, one study has shown that MALDI-TOF MS may be useful diagnostic tool for identifying isolates from patients with orthopaedic implant associated infections, providing reliable species level identification which may be used to differentiate between contaminates and pathogens (89, 90),(91).

6.4 Post-laboratory processes (post analytical stage)

6.4.1 Culture

Interpreting and reporting results

Following results should be reported:

- all organisms
- absence of growth

Intra-operative samples interpretation: 2 or more samples with an indistinguishable organism is indicative of a prosthetic joint infection.

Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Note: Due to extended incubation in certain situations, some final reports may not be available until greater than 14 days.

Also, report results of supplementary investigations.

Supplementary investigations: B 40 – Investigation of specimens for *Mycobacterium* species

6.4.2 Microscopy

Interpreting and reporting results

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

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.

Thresholds for white blood cell count and neutrophil percentage(35)

	Approximate cut off for acute PJI less than or equal to 6 week after most recent surgery	**Approximate cut off for acute PJI greater than 6 week after most recent surgery**
Synovial white blood cell count (WBC)	greater than 10,000 cells/µL	greater than 3,000 cells/µL
Synovial neutrophil percentage (PMN%)	greater than 90%	greater than 80%

6.4.3 Molecular

Report results as per manufacturer's instructions:

- detected
- not detected

6.5 Technical information and limitations

6.5.1 Sonication

Gram positive bacteria have been found to be resistant to the effect of ultrasound; Gram negative organisms may be more susceptible (58). 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 (9, 36, 92).

6.5.2 Small colony variants

Where primary plates are used they should be examined under magnification as small colony variants of staphylococci may be isolated from deep samples (63). Such small colonies may only become evident on prolonged culture (62). Thymidine dependent auxotrophs usually do not grow on blood agar and have atypical colonial appearance resembling haemophili or streptococci on chocolate agar (93). The true prevalence and clinical relevance of small colony forms in prosthetic joint infection is unclear (63, 94).

6.5.3 Contamination

Repetitive subculture from the enrichment broth during incubation may lead to contamination; the use of continuous monitoring blood culture bottles reduces this risk (21).

6.5.4 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 (42, 47, 48). One study comparing the culture of samples obtained by sonication of the prostheses and conventional periprosthetic-tissue culture has shown

that sensitivity of both culture methods is reduced in patients receiving antimicrobial therapy within 14 days before surgery (42). 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 (for example in the 30 to 60 minutes prior to skin incision) (94). The timing of prophylactic antibiotics is a risk-benefit decision.

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 2 stage revision. In some centres in selected cases however, one-stage revision is performed even in the presence of infection (95, 96).

7. Antimicrobial susceptibility testing

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

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory.

Extensive antibiograms (including rifampicin) on multiple isolates are required (32, 51, 60). 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 and linezolid.

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

8. Notification to PHE or equivalent in the devolved administrations

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 7 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 7 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(97, 98),(99 to 102).

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) and 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'.

Other arrangements exist in Scotland (99, 100)

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, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

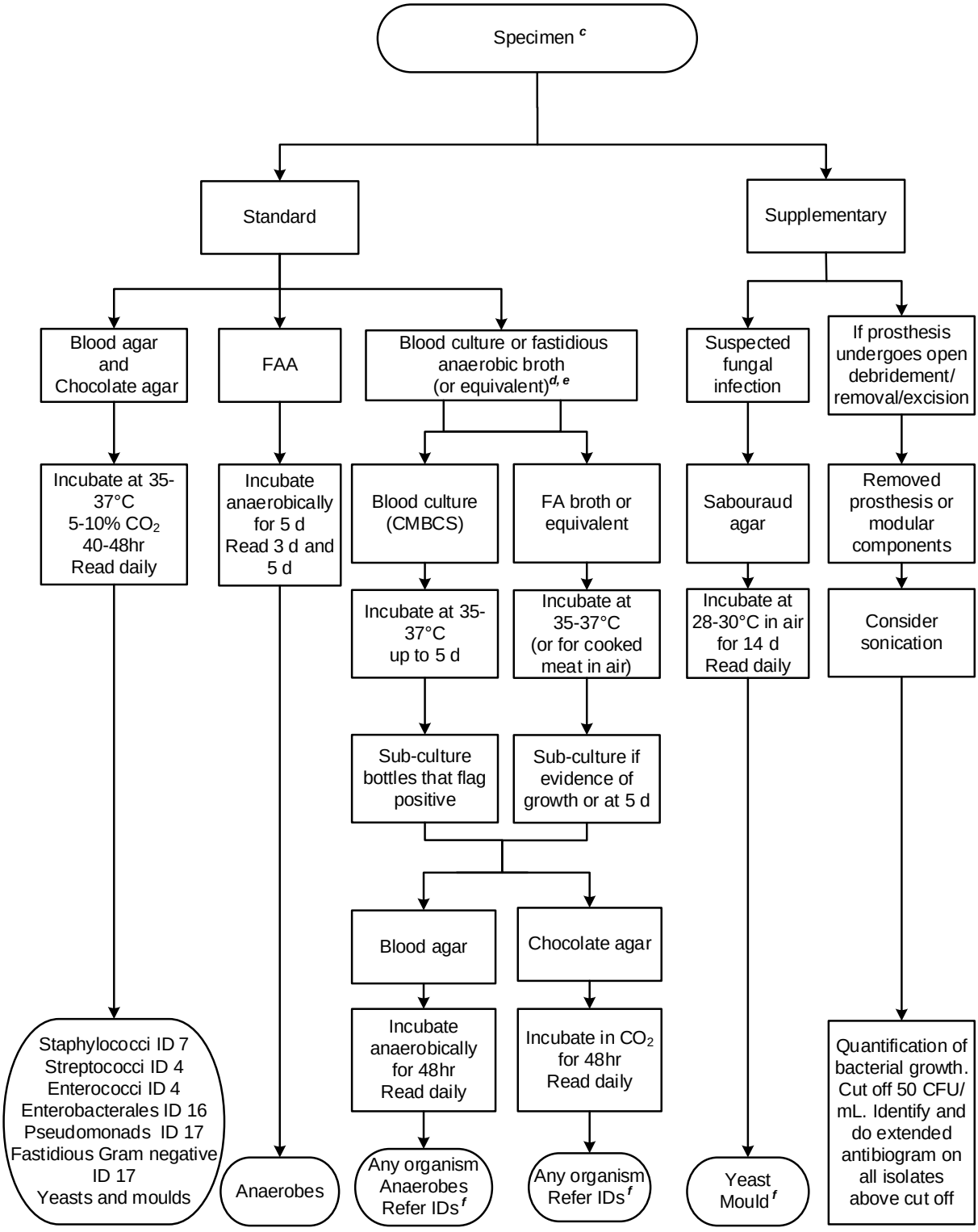
Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

- England and Wales
- Scotland
- Northern Ireland

Notes: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Appendix: Investigation of orthopaedic implant associated infections by culture[a, b]

A text description of this algorithm is provided with this document on the UK SMI B 44: investigation of orthopaedic implant associated infections webpage.



Footnotes

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

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

c) Mycobacterial cultures if any clinical suspicion such as 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) 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 (35).

f) Interpretation of intra-operative samples: 2 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, and others. Implant sonication for the diagnosis of prosthetic elbow infection. JShoulderElbowSurg. 2011;20(8):1275-81.
2. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, and others. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. JClinMicrobiol. 1998;36(10):2932-9.
3. 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. JInfect. 2007;55(1):1-7.
4. 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(4):185-211.
5. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, and others. Risk factors for prosthetic joint infection: case-control study. ClinInfectDis. 1998;27(5):1247-54.
6. Public Health England. Surveillance of surgical site infections in NHS hospitals in England 2013/2014. London; 2014 12/2014.
7. Lamagni T. Epidemiology and burden of prosthetic joint infections. JAntimicrobChemother. 2014;69 Suppl 1:i5-10.
8. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, and others. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. JOrthopRes. 2002;20(3):506-15.
9. Tande AJ, Patel R. Prosthetic joint infection. Clinical microbiology reviews. 2014;27(2):302-45.
10. National Institute for Health and Care Excellence. Surgical site infection: Prevention and treatment of surgical site infection. [Internet]. 2008.
11. National Institute for Health and Clinical Excellence. Quality Standards for Surgical Site Infection QS49. [Internet]. 2013.
12. Murdoch DR, Roberts SA, Fowler JV, Jr., Shah MA, Taylor SL, Morris AJ, and others. Infection of orthopedic prostheses after Staphylococcus aureus bacteremia. ClinInfectDis. 2001;32(4):647-9.
13. Marculescu CE, Berbari EF, Cockerill FR, III, Osmon DR. Unusual aerobic and anaerobic bacteria associated with prosthetic joint infections. ClinOrthopRelat Res. 2006;451:55-63.
14. Marculescu CE, Berbari EF, Cockerill FR, III, Osmon DR. Fungi, mycobacteria, zoonotic and other organisms in prosthetic joint infection. ClinOrthopRelat Res. 2006;451:64-72.
15. Gristina AG, Naylor P, Myrvik Q. Infections from biomaterials and implants: a race for the surface. MedProgTechnol. 1988;14(3-4):205-24.
16. Hoiby N, Bjarnsholt T, Moser C, Bassi GL, Coenye T, Donelli G, and others. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clinical microbiology and infection : the official publication of the European

Society of Clinical Microbiology and Infectious Diseases. 2015;21 Suppl 1:S1-25.
17. Esposito S, Leone S. Prosthetic joint infections: microbiology, diagnosis, management and prevention. IntJAntimicrobAgents. 2008;32(4):287-93.
18. Fernandez-Sampedro M, Salas-Venero C, Farinas-Alvarez C, Sumillera M, Perez-Carro L, Fakkas-Fernandez M, and others. 26Postoperative diagnosis and outcome in patients with revision arthroplasty for aseptic loosening. BMC infectious diseases. 2015;15:232.
19. Mackowiak PA, Jones SR, Smith JW. Diagnostic value of sinus-tract cultures in chronic osteomyelitis. JAMA. 1978;239(26):2772-5.
20. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. 2005 2005.
21. 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. ClinMicrobiolInfect. 2011;17(10):1528-30.
22. 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. DiagnMicrobiolInfectDis. 2010;68(1):83-5.
23. 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". Official Journal of the European Communities 1998;1-370 [Internet]. 1998.
24. 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. 1998 12/7/1998.
25. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 2009.
26. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
27. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. [Internet]. 2012
28. Home Office. Anti-terrorism, Crime and Security Act. 2001
29. Oethinger M, Warner DK, Schindler SA, Kobayashi H, Bauer TW. Diagnosing periprosthetic infection: false-positive intraoperative Gram stains. ClinOrthopRelat Res. 2011;469(4):954-60.
30. Johnson AJ, Zywiel MG, Stroh DA, Marker DR, Mont MA. Should gram stains have a role in diagnosing hip arthroplasty infections? ClinOrthopRelat Res. 2010;468(9):2387-91.
31. 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. AmJMed. 2004;117(8):556-62.

32. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, and others. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of america. ClinInfectDis. 2013;56(1):e1-e25.
33. Ghanem E, Parvizi J, Burnett RS, Sharkey PF, Keshavarzi N, Aggarwal A, and others. Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. JBone Joint SurgAm. 2008;90(8):1637-43.
34. Society of Unicondylar Research and Continuing Education. Diagnosis of Periprosthetic Joint Infection After Unicompartmental Knee Arthroplasty. 2012 9/2012. Report No.: 0883-5403 Contract No.: 8, Supplement.
35. Parvizi J, Gehrke T, Chen AF. Proceedings of the International Consensus on Periprosthetic Joint Infection. Bone Joint J. 2013;95-B(11):1450-2.
36. Gomez E, Patel R. Laboratory Diagnosis of Prosthetic Joint Infection, Part I. Clinical Microbiology Newsletter. 2011;33(8):55-60.
37. 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(4):554-7.
38. Parvizi J, Ghanem E, Sharkey P, Aggarwal A, Burnett RS, Barrack RL. Diagnosis of infected total knee: findings of a multicenter database. ClinOrthopRelat Res. 2008;466(11):2628-33.
39. Schinsky MF, Della Valle CJ, Sporer SM, Paprosky WG. Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. JBone Joint SurgAm. 2008;90(9):1869-75.
40. Athanasou NA, Pandey R, de Steiger R, Crook D, Smith PM. Diagnosis of infection by frozen section during revision arthroplasty. JBone Joint SurgBr. 1995;77(1):28-33.
41. Tsaras G, Maduka-Ezeh A, Inwards CY, Mabry T, Erwin PJ, Murad MH, and others. Utility of intraoperative frozen section histopathology in the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. JBone Joint SurgAm. 2012;94(18):1700-11.
42. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, and others. Sonication of removed hip and knee prostheses for diagnosis of infection. NEnglJMed. 2007;357(7):654-63.
43. Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelo J, Osmon DR, and others. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. JClinMicrobiol. 2009;47(6):1878-84.
44. Portillo ME, Salvado M, Trampuz A, Siverio A, Alier A, Sorli L, and others. Improved diagnosis of orthopedic implant-associated infection by inoculation of sonication fluid into blood culture bottles. Journal of clinical microbiology. 2015;53(5):1622-7.
45. Sorli L, Puig L, Torres-Claramunt R, Gonzalez A, Alier A, Knobel H, and others. 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. JBone Joint SurgBr. 2012;94(2):249-53.
46. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, Jr., and others. 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). ClinInfectDis. 2013;57(4):e22-e121.
47. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. NEnglJMed. 2004;351(16):1645-54.
48. Del Pozo JL, Patel R. Clinical practice. Infection associated with prosthetic joints. NEnglJMed. 2009;361(8):787-94.
49. Roux AL, Sivadon-Tardy V, Bauer T, Lortat-Jacob A, Herrmann JL, Gaillard JL, and others. Diagnosis of prosthetic joint infection by beadmill processing of a periprosthetic specimen. ClinMicrobiolInfect. 2011;17(3):447-50.
50. 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. JApplBacteriol. 1986;61(4):357-63.
51. Portillo E, Salvado M, Alier A, Martinez S, Sorli L, Horcajada JP, and others. Advantages of sonication fluid culture for the diagnosis of prosthetic joint infection. Journal of Infection. 2014;69(1):35-41.
52. Scorzolini L, Lichtner M, Iannetta M, Mengoni F, Russo G, Panni AS, and others. Sonication technique improves microbiological diagnosis in patients treated with antibiotics before surgery for prosthetic joint infections. New Microbiol. 2014;37(3):321-8.
53. Shen H, Tang J, Wang Q, Jiang Y, Zhang X. Sonication of explanted prosthesis combined with incubation in BD bactec bottles for pathogen-based diagnosis of prosthetic joint infection. Journal of clinical microbiology. 2015;53(3):777-81.
54. Mariconda M, Ascione T, Balato G, Rotondo R, Smeraglia F, Costa GG, and others. Sonication of antibiotic-loaded cement spacers in a two-stage revision protocol for infected joint arthroplasty. BMCMusculoskeletDisord. 2013;14:193.
55. Evangelopoulos DS, Stathopoulos IP, Morassi GP, Koufos S, Albarni A, Karampinas PK, and others. Sonication: a valuable technique for diagnosis and treatment of periprosthetic joint infections. TheScientificWorldJournal. 2013;2013:375140.
56. Esteban J, Alonso-Rodriguez N, del-Prado G, Ortiz-Perez A, Molina-Manso D, Cordero-Ampuero J, and others. PCR-hybridization after sonication improves diagnosis of implant-related infection. Acta Orthop. 2012;83(3):299-304.
57. 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. JOrthopRes. 2011;29(4):617-22.
58. 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. JClinMicrobiol. 2009;47(8):2496-501.
59. Musculoskeletal Infection Society. International consensus on periprosthetic joint infection. 2013.
60. 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. BMCInfectDis. 2014;14(1):233.
61. Wang B, Toye B, Desjardins M, Lapner P, Lee C. A 7-year retrospective review from 2005 to 2011 of Propionibacterium acnes shoulder infections in Ottawa, Ontario, Canada. Diagnostic microbiology and infectious disease. 2013;75(2):195-9.

62. Looney WJ. Small-colony variants of Staphylococcus aureus. BrJBiomedSci. 2000;57(4):317-22.
63. Maduka-Ezeh AN, Greenwood-Quaintance KE, Karau MJ, Berbari EF, Osmon DR, Hanssen AD, and others. Antimicrobial susceptibility and biofilm formation of Staphylococcus epidermidis small colony variants associated with prosthetic joint infection. DiagnMicrobiolInfectDis. 2012;74(3):224-9.
64. 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(1):37-40.
65. 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. JArthroplasty. 1999;14(8):952-6.
66. Zywiel MG, Stroh DA, Johnson AJ, Marker DR, Mont MA. Gram stains have limited application in the diagnosis of infected total knee arthroplasty. IntJInfectDis. 2011;15(10):e702-e5.
67. Font-Vizcarra L, Garcia S, Martinez-Pastor JC, Sierra JM, Soriano A. Blood culture flasks for culturing synovial fluid in prosthetic joint infections. ClinOrthopRelat Res. 2010;468(8):2238-43.
68. 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. BiomedResInt. 2014;2014:180416.
69. Penders J, Fiers T, Dhondt AM, Claeys G, Delanghe JR. Automated flow cytometry analysis of peritoneal dialysis fluid. NephrolDialTransplant. 2004;19(2):463-8.
70. Schafer P, Fink B, Sandow D, Margull A, Berger I, Frommelt L. Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. ClinInfectDis. 2008;47(11):1403-9.
71. 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. JMed Microbiol. 2012;61(Pt 3):309-16.
72. Butler-Wu SM, Burns EM, Pottinger PS, Magaret AS, Rakeman JL, Matsen FA, III, and others. Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. JClinMicrobiol. 2011;49(7):2490-5.
73. Levy PY, Fenollar F, Stein A, Borrione F, Cohen E, Lebail B, and others. Propionibacterium acnes postoperative shoulder arthritis: an emerging clinical entity. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;46(12):1884-6.
74. Butler-Wu SM, Cookson BT. Reply to "Anaerobic thioglycolate broth culture for recovery of propionibacterium acnes from shoulder tissue and fluid specimens". Journal of clinical microbiology. 2013;51(2):733.
75. Shannon SK, Mandrekar J, Gustafson DR, Rucinski SL, Dailey AL, Segner RE, and others. Anaerobic thioglycolate broth culture for recovery of Propionibacterium acnes from shoulder tissue and fluid specimens. Journal of clinical microbiology. 2013;51(2):731-2.
76. Schwotzer N, Wahl P, Fracheboud D, Gautier E, Chuard C. Optimal culture incubation time in orthopedic device-associated infections: a retrospective analysis of prolonged 14-day incubation. Journal of clinical microbiology. 2014;52(1):61-6.

77. Bosshard PP. Incubation of fungal cultures: how long is long enough? Mycoses. 2011;54(5):e539-e45.
78. 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. JMedMicrobiol. 1996;44(5):355-61.
79. Arciola CR, Montanaro L, Costerton JW. New trends in diagnosis and control strategies for implant infections. IntJArtifOrgans. 2011;34(9):727-36.
80. Cazanave C, Greenwood-Quaintance KE, Hanssen AD, Karau MJ, Schmidt SM, Gomez Urena EO, and others. Rapid molecular microbiologic diagnosis of prosthetic joint infection. JClinMicrobiol. 2013;51(7):2280-7.
81. Gomez E, Cazanave C, Cunningham SA, Greenwood-Quaintance KE, Steckelberg JM, Uhl JR, and others. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. JClinMicrobiol. 2012;50(11):3501-8.
82. Zegaer BH, Ioannidis A, Babis GC, Ioannidou V, Kossyvakis A, Bersimis S, and others. Detection of Bacteria Bearing Resistant Biofilm Forms, by Using the Universal and Specific PCR is Still Unhelpful in the Diagnosis of Periprosthetic Joint Infections. Front Med (Lausanne). 2014;1:30.
83. Hartley JC, Harris KA. Molecular techniques for diagnosing prosthetic joint infections. JAntimicrobChemother. 2014;69 Suppl 1:i21-i4.
84. Dempsey KE, Riggio MP, Lennon A, Hannah VE, Ramage G, Allan D, and others. Identification of bacteria on the surface of clinically infected and non-infected prosthetic hip joints removed during revision arthroplasties by 16S rRNA gene sequencing and by microbiological culture. Arthritis ResTher. 2007;9(3):R46.
85. Rak M, Barlic-Maganja D, Kavcic M, Trebse R, Cor A. Comparison of molecular and culture method in diagnosis of prosthetic joint infection. FEMS microbiology letters. 2013;343(1):42-8.
86. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, and others. Real-time PCR in clinical microbiology: applications for routine laboratory testing. ClinMicrobiolRev. 2006;19(1):165-256.
87. Cherkaoui A, Ceroni D, Emonet S, Lefevre Y, Schrenzel J. Molecular diagnosis of Kingella kingae osteoarticular infections by specific real-time PCR assay. JMed Microbiol. 2009;58(Pt 1):65-8.
88. Bjerkan G, Witso E, Nor A, Viset T, Loseth K, Lydersen S, and others. A comprehensive microbiological evaluation of fifty-four patients undergoing revision surgery due to prosthetic joint loosening. JMed Microbiol. 2012;61(Pt 4):572-81.
89. Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti JL, and others. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. ClinBiochem. 2011;44(1):104-9.
90. 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–¿. JClinMicrobiol. 2010;48(3):900-7.
91. Peel TN, Cole NC, Dylla BL, Patel R. Matrix-assisted laser desorption ionization time of flight mass spectrometry and diagnostic testing for prosthetic joint

infection in the clinical microbiology laboratory. Diagnostic microbiology and infectious disease. 2015;81(3):163-8.
92. Del AA, Bertrand ML. The diagnosis of periprosthetic infection. OpenOrthopJ. 2013;7:178-83.
93. Gilligan PH, Gage PA, Welch DF, Muszynski MJ, Wait KR. Prevalence of thymidine-dependent Staphylococcus aureus in patients with cystic fibrosis. JClinMicrobiol. 1987;25(7):1258-61.
94. Geipel U. Pathogenic organisms in hip joint infections. IntJMed Sci. 2009;6(5):234-40.
95. Gehrke T, Zahar A, Kendoff D. One-stage exchange: it all began here. The bone & joint journal. 2013;95-b(11 Suppl A):77-83.
96. Achermann Y, Stasch P, Preiss S, Lucke K, Vogt M. Characteristics and treatment outcomes of 69 cases with early prosthetic joint infections of the hip and knee. Infection. 2014;42(3):511-9.
97. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013 7/2013.
98. Department of Health. Health Protection Legislation (England) Guidance. 1-112. 2010.
99. Scottish Government. Public Health (Scotland) Act. 2008. Available from: 2008 (as amended).
100. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States.; 2009 2009.
101. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.
102. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. [Internet]. 1967.