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Health Protection Agency

NATIONAL STANDARD METHOD

INVESTIGATION OF PROSTHETIC JOINT INFECTION SAMPLES

BSOP 44

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

Scottish Microbiology Forum

Association of Medical Microbiologists

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AMENDMENT PROCEDURE

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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
1/ 11.12.09	1	1.1		**Front page**	SMF logo added
			7 10	**Technical Information/Limitations** **1.2 Specimen transport and storage**	The term "CE Marked leak proof container" replaces "sterile leak proof container"; endnote[a] added to clarify the change; reference[43] inserted to the IVD Directive 98/79/EC.

INVESTIGATION OF PROSTHETIC JOINT INFECTION SAMPLES

Types of specimen: Prosthetic joint aspirate
Peri-prosthetic biopsy
Intra-operative specimens (debridement and retention or revision surgery)
Prostheses

SCOPE OF DOCUMENT

This National Standard Methods (NSM) describes the processing and bacteriological investigation of prosthetic joint infections. For information on bone samples refer to BSOP 42 – Investigation of bone and soft tissue associated with osteomyelitis.

INTRODUCTION

Since the earliest hip replacements, pioneered by Sir John Charnley in the early 1960s, joint replacement (arthroplasty) has become a common procedure. It is done most commonly for osteoarthritis and inflammatory arthopathies such as rheumatoid arthritis. For hip fractures, a hemiarthroplasty is one of the early surgical treatment options. Hip and knee replacements are more common than replacements of shoulder, elbow, ankle and interphalangeal joints. Spinal disc replacements are a very recent introduction still at the developmental stage. Bilateral replacements for osteoarthritis are common in weight bearing joints and multiple joint replacements are common in inflammatory arthritis. Multiple replacements carry the important implication that local symptoms and localised microbiological diagnosis of infection are critical in determining which joint is subjected to revision arthroplasty. It is important to note that other complications are more common causes of the need for joint revision than infection and this has been true since the earliest days of arthroplasty[2]. In one series only some 14% of revision surgery was performed for infection[3].

With modern surgical and anaesthetic techniques, appropriate patient selection, modern prosthesis design, prophylactic antibiotics, ultraclean laminar airflow in operating theatres and good post-operative care, infection rates are now much lower than when joint replacement was first introduced. However there is still a finite risk associated with each procedure. This is around 1% for elective hip and knee replacements and 4% for emergency hemiarthroplasties[4]. The risk of infection in a joint replacement is increased by patient co-morbidities, including the early development of a surgical site infection not apparently involving the prosthesis, a National Nosocominal Infections Surveillance Score of 1 or 2, the presence of malignancy and previous joint arthroplasty[5]. 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].

Organisms may be introduced into the joint, establishing acute or chronic infection, during primary implantation surgery or the haematogenous (bloodstream) route. Fewer organisms are required to establish infection when there is a foreign body *in situ* than otherwise[7]. It is estimated that up to 30% of *S. aureus* bacteraemias are associated with septic arthritis in those with pre-existing prosthetic joints[8]. The most common organism to cause acute infections is *Staphyloccus aureus (*meticillin sensitive or resistant) and in chronic infections either *S. aureus* or coagulase negative staphylococci. Many other organisms can be acquired by either direct inoculation or the haematogenous route including other skin flora, streptococci, coliforms, enterococci and rarely anerobes, mycobacteria or fungi[9-11]. This means that any organism cultured from a sample associated with a prosthetic joint or other orthopaedic device could be significant. It is for this reason that multiple samples should be taken.

Once infection is estalished around a prosthetic joint, organisms can form a 'biofilm'[12]. 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 antimicrobial sensitivities performed by standard methods may not predict the required antimicrobial activity. *In vitro* models testing activity of antimicrobials against biofilm organisms are not at present feasible in routine laboratories.

Prosthetic joint infections can present acutely, usually with a hot, swollen painful joint. The patient is often febrile and can be clinically septic. Inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are usually markedly raised. This presentation needs to be differentiated from acute inflammatory arthritides such as rheumatoid arthritis, gout, pseudogout and also from an acute haematoma (blood) in the joint. Alternatively, prosthetic joint infections can present chronically. The joint may simply be painful and stiff. There may be evidence for loosening of the prosthesis on X-ray. Inflammatory markers may be slightly raised, but this is non-specific. These presentations are often difficult to differentiate from those of mechanical pain or aseptic loosening, whereas the presence of a discharging sinus indicates the presence of a deep prosthetic joint infection. Ultimately many painful, loose prostheses require surgical revision (exchange). Of patients undergoing elective revision around 14% are found to be infected[3].

In the acute presentation of prosthetic joint infection, in addition to a full clinical assessment of the patient, blood cultures should be taken and a joint aspirate performed if possible. Synovial fluid may be visibly purulent or merely turbid. Plain X-rays are performed to rule out fracture and to look for evidence of infection. In the chronically infected prosthetic joint, the diagnosis is much more difficult. A past history of early post-operative wound infection increases the likelihood of deep infection. Plain X-rays may show loosening but this does not differentiate septic from aseptic loosening. If changes are rapidly progressive over time, infection is more likely. Nuclear radiology may have a role in diagnosis but scans can be non-specific or technically difficult to perform. MRI and CT are rarely helpful. Inflammatory markers may only be slightly raised and are not specific or sensitive. Sinus cultures are not helpful as organisms cultured do not predict those causing deep infection[13]. A joint aspirate or periprosthetic joint biopsy for microbiology and histology are the most specific tests for infection. As organisms may be in a 'sessile' biofilm form rather than 'planktonic' and loose in the joint fluid, the sensitivity of a joint aspirate, however, can be poor. A joint aspirate can be performed on the ward, in radiology departments or in theatre, at the discretion of the orthopaedic surgeon who should always be involved in management decisions.

In the absence of radiological or clinical evidence for loosening and with a short duration of symptoms, some selected patients can be managed with early prosthesis debridement and implant retention. If possible this should be done before the patient receives antibiotics, or at least with a pre-operative aspirate obtained off antibiotics. In theatre several samples should be taken for microbiology and if the presence of infection is not clear (eg if there is no obvious purulence), also for histology. As organisms are likely to be in biofilm on the retained prosthesis, antibiotics that have activity against organisms in this growth mode should be used where possible. For staphylococcal infection, rifampicin combinations may be the most effective [14-16]. Other antibiotics that may be used orally, often in combination with rifampicin (which of course cannot be used in monotherapy because of the risk of development of antimicrobial resistance), are quinolones, fusidic acid, tetracyclines such as doxycycline or minocycline, and co-trimoxazole[17]. Occasionally, Linezolid, quinupristin-dalfopristin and other agents may be used.

In cases where a prosthetic joint is chronically painful and loose, but the presence of infection is not known, an elective revision may be performed. When there is no pre-operative suspicion of infection, revision of the joint in one sitting is recommended. After opening the joint, multiple (4-5) samples should be taken from different sites for microbiology and equivalent samples taken for histology. A risk-benefit assessment of antibiotic timing is required. Where infection is likely and/or a microbiological diagnosis is likely to significantly affect clinical outcome, prophylactic antibiotics can be witheld until immediately after sampling. When a tourniquet is used, antibiotics should be administered before inflation. The effect of a single dose of antibiotic on the sensitivity of microbiological culture is unknown. It is important for microbiological culture that separate instruments are used for each sample to prevent cross contamination of samples. In some equivocal cases, where

available, frozen section for histology can be done, only proceeding to re-implantation if this shows no evidence for infection.

In patients with a chonically infected joint, either discovered at routine revision, or diagnosed by the presence of a sinus or microbiological tests, the preferred option in many centres is to remove the joint and do a thorough debridement without immediate re-implantation. In some centres, one-stage revision is performed even in the presence of infection. Again, multiple samples should be taken, as described above. In some cases (especially in infected knee replacements) an antibiotic-loaded cement spacer is put in to protect the joint integrity and avoid impaction of debrided bone ends. Commercially available cements contain antibiotics such as gentamicin or tobramycin. Post-operatively, patients generally receive broad spectrum antibiotics until microbiological results are available. Definitive therapy is usually for several weeks until there is good evidence that the wound is healed and inflammatory markers have normalised. If re-implantation is planned this is performed at this stage or any time afterwards.

TECHNICAL INFORMATION/LIMITATIONS

In National Standard Methods, the term "CE marked leak proof container" is used to describe containers bearing the CE marking and which are used for the collection and transport of clinical specimens. The requirements of the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1)[18] state that such devices must "reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Percutaneous joint aspiration

This is an important diagnostic test in both acute and chronic prosthetic joint infections. It is important that this is performed aseptically, ideally in radiology or in theatres. In acute infections, a Gram stain is useful although a negative result should not rule out the possibility of infection. A semi-quantitative white cell count on the synovial fluid is useful for differentiating inflammatory from non-inflammatory arthritides, however is less useful at differentiating infection from inflammation[3]. In the latter, crystals should be searched for in the synovial fluid. A quantitative and differential white cell count may be helpful in patients with underlying osteoarthritis. In one study a leukocyte count of >1.7 X 10^3/uL had a sensitivity of 94% and specificity of 88% for diagnosing prosthetic infection compared with aseptic loosening. The authors however excluded all patients with an underlying inflammatory arthropathy[19].

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

Percutanous biopsy

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

Intra-operative biopsies

Intra-operative biopsies may be performed in the chronically infected joint either solely as a diagnostic test, as part of a debridement and retention procedure, or when a joint is being revised. Joint revision is a common procedure and usually done for aseptic loosening. However, because infection can be occult, it is advisable to take multiple samples for microbiology and histology in all cases. In some cases, where available, this can be combined with a frozen section to aid decision making[20].

Samples should be taken early in the procedure, just prior to administering prophylactic antibiotics where infection is likely and/or a microbiological diagnosis is likely to significantly affect outcome. When a tourniquet is used, antibiotics should be administered before inflation. Samples of fluid, pus, synovium, granulation tissue and any abnormal areas should be taken, particularly from the peri-prosthetic "membranes", (the tissue that forms at the bone-cement or bone-prosthesis interfaces), in cases where the joint is being removed. Each specimen should be taken with a separate set of instruments, and should be placed into a separate specimen container. Pre-sterilised packs can be produced for this purpose. At this stage a frozen section may also be performed if available and required to decide between one- and two-stage exchange.

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

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

Samples can be transferred to the laboratory using routine timescales (eg within hours rather than minutes). There are no published comparisons or validations of various tissue processing methods in the orthopaedic setting. Shaking with Ballotini beads is relatively simple and therefore carries a low risk of contamination. This method of tissue disruption has been shown experimentally to be superior to shaking in broth alone in the recovery of bacillus spores from polymer surfaces[21].

Sonication has been examined in the research setting as a means of disrupting bacterial biofilm in vascular and orthopaedic prostheses. Clinical studies of sonication in orthopaedics have, until recently, been fraught with practical difficulties and specimen bag leakage. A recent study appears to have overcome the risk of leakage by using specimen pots large enough to accommodate the prosthesis, however sonication of orthopaedic samples remains a technique for the clinical research setting at present[22].

Gram staining in elective revision cases has extremely poor sensitivity. It has a useful role in acute infections. Organisms can be cultured from 60 - 70% of samples taken from prostheses deemed to be infected (using histology as a surrogate criterion standard)[3]. As the organisms that cause chronic prosthetic joint infection are frequently the same as those that contaminate microbiological samples, interpretation of results is difficult when only one or two samples are taken. At least 4 to 5 samples are recommended. When 5 samples are taken, the false positive rate with 2 or 3 samples positive is <5% whereas false positive rates close to 30% are seen with a single positive sample. 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. Obtaining organisms from a single tissue sample therefore poses significant challenges in interpretation. Even with careful sampling and prolonged cultures, there is still a significant culture negative rate, even when histology is positive. This may be due to sampling error (the distribution of organisms can be patchy), very small numbers of organisms that do not thrive in laboratory culture conditions, an inability to disrupt organisms from the biofilm, unculturable organisms or false positive histology results. Immuno-fluorescent and molecular studies suggest that, in some cases, there may be organisms present even when conventional cultures are negative[3].

The culture methodology that has been validated[3] by comparison with histology involves liquid culture and prolonged incubation of both primary and subculture plates. No comparative culture methodologies have been evaluated and it is not clear which components of the described methodology are critical. It may not be important in elective revisions to include plates, provided multiple sites are sampled and put into broth media. Work with *S. epidermidis* and vascular grafts suggests that liquid enrichment is as important as sonication or grinding[23]. Exclusion of contaminants during operative and laboratory processing is important.

Examination of culture plates with a plate microscope may be important because small-colony variants of staphylococci may be isolated from deep samples. Such small-colony variants may emerge on vancomycin therapy[24], although this particular description relates to catheter associated infections. Gentamicin and co-trimoxazole use are more frequently associated with emergence of small colony auxotrophs for thymidine, menadione, or haemin[25]. Such small colonies may only become evident on prolonged culture [26]. Thymidine dependent auxotrophs usually do not grow on blood agar and have atypical colonial appearance resembling Haemophili or streptococci on chocolated agar[27]. The true prevalence of small colony forms in prosthetic joint infection in cemented prostheses is unclear. The organisms may be present in areas near the prosthesis with low concentrations of antibiotic diffusing from the cement.

Defining organisms in separate samples as indistinguishable can be difficult. One or two differences in an extended antibiogram may not always indicate strains from different clonal origins. In addition, infection of prostheses with multiple strains can occur[3]. It is important to perform sensitivity testing on all isolates from all samples as the presence of resistant strains will affect the outcome of therapy, and the extended antibiogram is a common and cheap way to identify strains as indistinguishable in multiple cultures.

Explanted prostheses

Explanted prostheses can be sent for microbiological investigation. They are often difficult to handle unless especially large pots are used (see sonication above) leading to a potentially greater risk of contamination.

Serological techniques

Serological techniques used for diagnosis of prosthetic joint infection have been studied in the research setting but have not been found to be of practical clinical use as yet. The problem tends to be with specificity[28]. Measurement of IgM antibodies in patients with vascular graft infections has also been studied although this is not in routine clinical use[29].

Molecular methods

Preliminary assessments of molecular methods applied to tissues to date suggests that the techniques are less sensitive than culture. Comparative validation of culture without liquid enrichment, direct immuno-fluorescence for coagulase negative staphylococci and propionibacteria, and 16S rDNA PCR on material dislodged from resected and transported prostheses suggested sensitivities of 22%, 63% and 72% respectively in one study[30]. PCR for 16S rDNA may have a clinical role in culture negative cases, but in general molecular methods are not yet ready for routine clinical management. Further studies are required to evaluate molecular methods, and validate them against robust clinical and conventional pathological definitions of infection.

1 SAFETY CONSIDERATIONS[4,31-41]

1.1 SPECIMEN COLLECTION

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

1.2 SPECIMEN TRANSPORT AND STORAGE

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

1.3 SPECIMEN PROCESSING

Containment Level 2 unless infection with a Hazard Group 3 organism is suspected on clinical grounds, for example tuberculosis, in which case work should be performed in a microbiological safety cabinet under Containment Level 3 conditions.

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

Refer to current guidelines on the safe handling of all organisms documented in this NSM.

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

Compliance with postal and transport regulations is essential.

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME FOR SPECIMEN COLLECTION

Before antimicrobial therapy where possible. Antibiotic prophylaxis for surgery should be withheld until samples have been taken.

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

The specimen will usually be collected by a specialist orthopaedic surgeon or in some cases by an interventional radiologist. CE Marked leak proof container[a] in a sealed plastic bag. Swabs should be discouraged unless there is no alternative.

Aseptic collection is important to avoid carry-over of contaminants. Direct collection in theatres into a CE Marked leak proof container[a] in a sealed plastic bag, with Ringer's or saline and Ballotini beads, may be preferable to a two stage process involving sample manipulation in the laboratory. Ringer's or saline should be substituted for molecular grade water if samples are to be examined by molecular methods.

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

For aspirates and radiologically guided biopsies, it is usually only possible to send one sample to microbiology. In theatres, multiple (4-5 samples) should be taken using separate instruments for microbiology. An equivalent set of samples should be taken for histology.

Specimen size should approximate to 1 mL.

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

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

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

N/A

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

(BSOPTP 39 - Staining Procedures)

4.3.1 STANDARD

Gram stain

Note: This is an insensitive procedure and not recommended for the pre or intra-operative diagnosis of chronic prosthetic joint infection. It does however have a role in acute prosthetic joint infection especially on a purulent aspirate or surgical pus. It is important to distinguish between aggregates of ultrasound-dislodged biofilm bacteria from other debris and contaminating bacteria. These can appear as odd single cells or very small groups of cells**.** A negative Gram stain does not rule out infection.

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

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

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

Alternatively, samples may be sent to the laboratory in CE Marked leak proof container[a] in a sealed plastic bag with no Ballotini beads. Ballotini beads and Ringer's or saline can be added in the laboratory, maintaining asepsis diligently. Clean air provision may be desirable. Homogenisation with Ballotini beads can be performed by shaking at 250 rpm for 10 minutes in a covered rack on an orbital shaker or, alternatively, vortexing for 15 seconds (40Hz).

The diluent for the Ballotini beads and tissues should be Ringer's or saline. Sterile molecular grade water and new universal containers should be used if direct PCR assays are planned. The volume used in the latter case should not exceed 2 mL to maintain assay sensitivity.

4.4.2 SPECIMEN PROCESSING

Soft tissue homogenate

Inoculate plates and broth after homogenisation. Inoculate each agar plate with a drop of the solution using a sterile pipette (see QSOP 52 - Inoculation of Culture Media). 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.

Incubate the enrichment broth for a further five days, examining daily for turbidity. Subculture if cloudy but otherwise perform a terminal subculture at five days. See table 4.4.3 for primary and subculture plates, broths, atmospheres and duration of cultures.

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

Primary plates should be examined with a plate microscope for small-colony variants. Care should be taken to distinguish small tissue fragments on the plate from small colonies. Small colony variants are often thymidine-dependent, at least if the patient has received co-trimoxazole. Such isolates may not grow well on horse blood agar due to partial lysis and release of thymidine phosphokinase from the red cells. Chocolating destroys thymidine phosphokinase.

4.4.3 CULTURE MEDIA, CONDITIONS AND ORGANISMS FOR ALL SPECIMENS

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Primary plates may not be needed in elective revisions in high volume units and skilled multiple site sampling.	Blood agar and chocolate agar	35 - 37	5 - 10% CO_2	2 days	Daily	Staphylococci Streptococci Enterococci Enterobacteriaceae Fastidious Gram negatives Pseudomonads Fungi
	Fastidious anaerobic agar	35 - 37	Anaerobic	5 days	48 h	Anaerobes
	*Fastidious anaerobic broth, cooked meat broth or equivalent Subculture when cloudy or at day 5 on plates as below	35 - 37	5 - 10% CO_2 or for Cooked meats – in air	5 days	N/A	Any
	Subculture plates: Blood agar – incubated anaerobically for 48 hours Sabouraud' s agar	35-37 30	Anaerobic Air	2 days 14 days	Daily	Any
	Chocolate agar incubated for 48 hours	35 - 37	5 – 10 % CO_2	2 days	Daily	Any

* Subcultures should be performed when the broth looks cloudy, terminal subcultures should be performed at 5 days

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

4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL OF IDENTIFICATION IN THE LABORATORY

Actinomycetes	genus level BSOPID 15 – Idetification of anaerobic *Actinomycetes* species
Anaerobes	genus level BSOPID 14 - Identification of non-sporing, non-branching anaerobes BSOPID 8 - Identification of Clostridium species BSOPID 25 - Identification of anaerobic Gram-negative rods
β-haemolytic streptococci	Lancefield group level
Other streptococci	species level
Enterococci	species level
Enterobacteriaceae	species level
Fungi	species level
Haemophilus species	species level
Pseudomonads	species level
S. aureus	species level
Staphylococci (not aureus)	usually genus level
Mycobacterium species	BSOP 40 - Investigation of specimens for Mycobacterium species

Note 1: Subculture after homogenisation, which is likely to generate aerosols, must be performed in a safety cabinet[34].

Note 2: No organism should be considered to be a contaminant until cultures on all samples are concluded. Identification to species level and/or an extended antibiogram is normally necessary to detect whether isolates from multiple samples are indistinguishable.

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

4.5.2 REFERRAL TO REFERENCE LABORATORIES

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

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

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

It is important to include a wide range of antibiotics particularly for those patients who may require prolonged oral treatment with biofilm active drugs (see introduction above). These antibiotics are not usually included in the common first line antibiotics tested in most laboratories. On Gram positive isolates these may include a teicoplanin MIC plus antibiotics such as rifampicin, tetracyclines, quinolones, co-trimoxazole, fusidic acid, linezolid, quinupristin/dalfopristin and others.

5 REPORTING PROCEDURE

5.1 MICROSCOPY

N/A

5.2 CULTURE

Report all organisms.

Report absence of growth.

Also, report results of supplementary investigations.

5.2.1 CULTURE REPORTING TIME

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

Supplementary investigations: BSOP 39 - Investigation of dermatological specimens for superficial mycoses, and BSOP 40 - Investigation of specimens for Mycobacterium species.

Clinically urgent results: telephone when available.

5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated.

6 REPORTING TO THE HPA[42] (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications:

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

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

Refer to local guidelines on CDSC and COSURV reporting

Local guidelines

7 ACKNOWLEDGEMENTS AND CONTACTS

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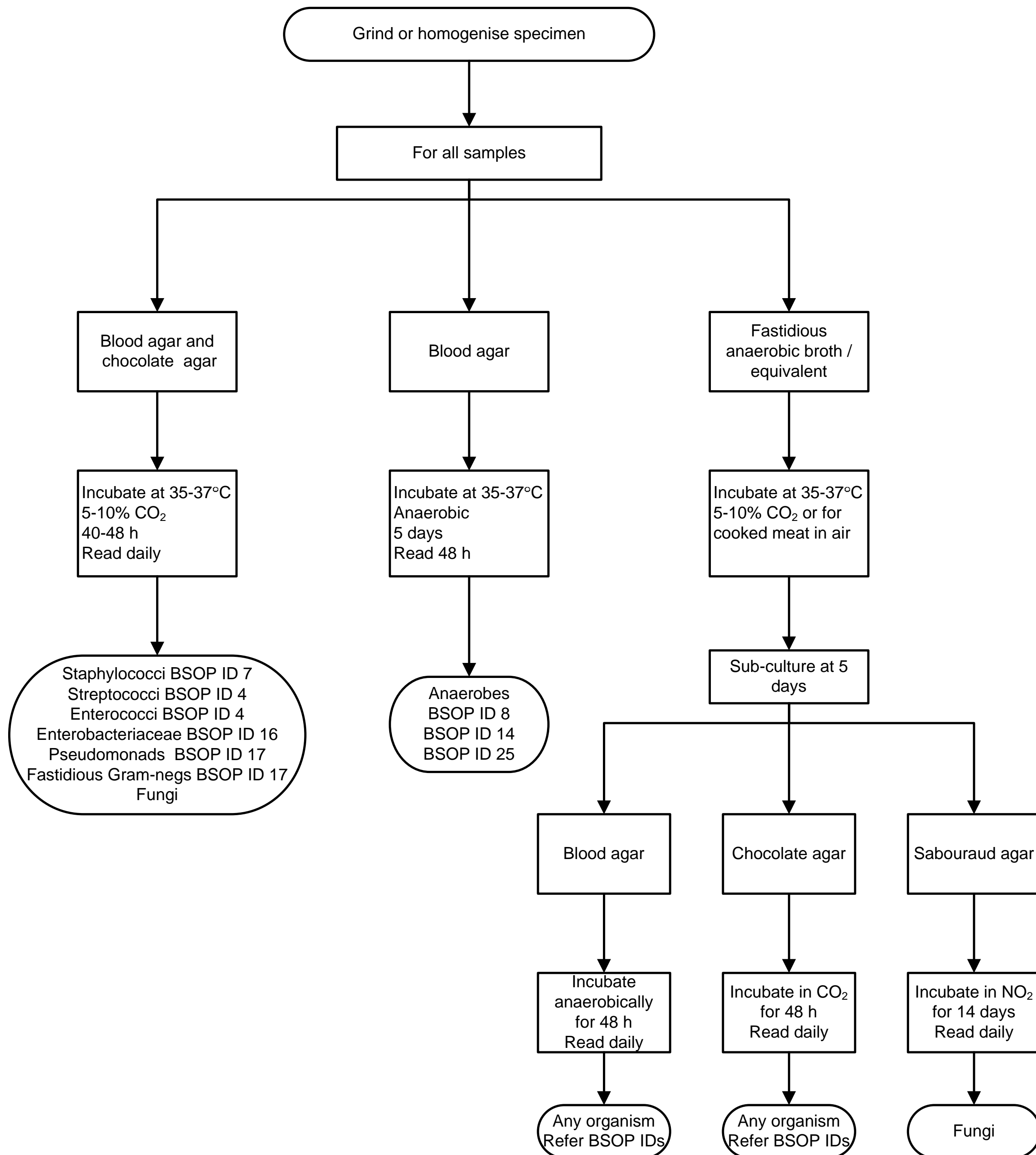
APPENDIX



REFERENCES

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

2. Salvati EA, Wilson PD, Jr., Jolley MN, Vakili F, Aglietti P, Brown GC. A ten-year follow-up study of our first one hundred consecutive Charnley total hip replacements. J Bone Joint Surg Am 1981;63:753-67.

3. 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.

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

5. 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.

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. ELEK SD, CONEN PE. The virulence of Staphylococcus pyogenes for man; a study of the problems of wound infection. Br J Exp Pathol 1957;38:573-86.

8. 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.

9. 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.

10. 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.

11. 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.

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

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

14. Zimmerli W, Frei R, Widmer AF, Rajacic Z. Microbiological tests to predict treatment outcome in experimental device-related infections due to Staphylococcus aureus. J Antimicrob Chemother 1994;33:959-67.

15. Widmer AF, Gaechter A, Ochsner PE, Zimmerli W. Antimicrobial treatment of orthopedic implant-related infections with rifampin combinations. Clin Infect Dis 1992;14:1251-3.

16. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA 1998;279:1537-41.

17. Stein A, Bataille JF, Drancourt M, Curvale G, Argenson JN, Groulier P, et al. Ambulatory treatment of multidrug-resistant Staphylococcus-infected orthopedic implants with high-dose oral co-trimoxazole (trimethoprim-sulfamethoxazole). Antimicrob Agents Chemother 1998;42:3086-91.

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

19. 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.

20. 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.

21. 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.

22. 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.

23. Bergamini TM, Bandyk DF, Govostis D, Vetsch R, Towne JB. Identification of Staphylococcus epidermidis vascular graft infections: a comparison of culture techniques. J Vasc Surg 1989;9:665-70.

24. Adler H, Widmer A, Frei R. Emergence of a teicoplanin-resistant small colony variant of Staphylococcus epidermidis during vancomycin therapy. Eur J Clin Microbiol Infect Dis 2003;22:746-8.

25. Proctor RA, Kahl B, von Eiff C, Vaudaux PE, Lew DP, Peters G. Staphylococcal small colony variants have novel mechanisms for antibiotic resistance. Clin Infect Dis 1998;27 Suppl 1:S68-S74.

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

27. 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.

28. 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.

29. Selan L, Passariello C, Rizzo L, Varesi P, Speziale F, Renzini G, et al. Diagnosis of vascular graft infections with antibodies against staphylococcal slime antigens. Lancet 2002;359:2166-8.

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

31. Health and Safety executive, editor. Biological agents: Managing the risks in laboratories and healthcare premises 5A.D. 2008.

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

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

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

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

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

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

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

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

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

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

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

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[a] *The requirements of the EU in vitro Diagnostic Medical Devices Directive[18] (98/79/EC Annex 1 B 2.1) state that such devices must "reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".*