Infection is a dire complication affecting every field of orthopaedics and traumatology. If specific clinical, laboratory and imaging parameters are present, infection is often assumed even in the absence of microbiological confirmation. However, apart from confirming infection, knowing the exact infecting pathogen(s) and their antimicrobial susceptibility patterns is paramount to help guide treatment. Every effort should therefore be undertaken with that goal in mind.

Not all microbiological findings carry the same relevance, and knowing exactly how and where a sample was collected is key. Several different sampling techniques are available, and one must be aware of both advantages and limitations. Microbiological sampling alternatives in some of the most common clinical scenarios such as native and prosthetic joint infections, osteomyelitis and fracture-related infections, spinal and diabetic foot infections will be discussed.

Orthopaedic surgeons should also be aware of basic laboratory sample processing techniques as they have a direct impact on the way specimens should be dealt with and transported to the laboratory. Only by knowing these basic principles will surgeons be able to participate in the multidisciplinary discussion and decision making around how to interpret microbiological findings in each specific patient.

Keywords: bone and joint infections; microbiology; tissue sampling

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Introduction

Infection is a serious complication that affects all fields of orthopaedics and traumatology. Even though in certain specific conditions infection can be assumed even in the absence of isolated pathogens, adequate microbiological confirmation is warranted in most clinical scenarios. Conversely, not all microbiological findings are necessarily pathologic as each individual is home to trillions of microbes that inhabit our bodies and constitute a healthy microbiome that contributes to normal homeostasis.1

Human skin protects the body from outside pathogens but is also home to a rich microbial community of its own, most notably Corynebacterium, Propionibacteriaceae and Staphylococci.2 Although in normal physiological conditions these bacteria are considered non-pathogenic, after operative procedures where the skin is breached, such as orthopaedic surgery, they are also among the most commonly found infective microorganisms.3

This scenario is aggravated by the presence of implants, such as screws, plates or even artificial joints that are frequently utilized in orthopaedic and trauma surgery. It is known that the presence of a foreign body reduces the amount of bacterial inoculum required to cause infection by a factor of more than 10^5.4 Biofilm formation on the surface of such implants is not only responsible for increased susceptibility but also for increased difficulty in isolating infecting microorganisms. Bacteria present within biofilms are not as easily retrieved or grown in the laboratory.3

When deciding how to interpret microbiological findings, a number of other factors such as underlying clinical scenario, presence or absence of other laboratory or imaging findings indicative of infection, previous antibiotic therapy, etc., should be considered. However, precise identification of the microorganism(s) causing infection is at the very least needed to allow for the selection of the narrowest spectrum, least toxic, preferably oral antibiotics.

A comprehensive knowledge of how the sample was collected and subsequently processed is a critical part of the multidisciplinary decision process and one of which all orthopaedic surgeons must be aware. The aim of this article is to review the best practices for obtaining adequate samples in the most frequent clinical scenarios:
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(a) native and prosthetic joint infections; (b) osteomyelitis and fracture-related infections; (c) spinal infections and; (d) diabetic foot infections. Although a thorough description of laboratory procedures is beyond the scope of this article, a short overview with a special emphasis on its impact on the correct collection and transport of specimens for microbiological investigation will also be provided.

Native and prosthetic joint infection

Whenever there is an infection, samples should be gathered as close to the site as possible. In the case of native or prosthetic joint infections (PJI), synovial fluid obtained through arthrocentesis of the affected joint is the best diagnostic sample. It allows not only for microbiological investigation but also more comprehensive investigation including differential leukocyte count and a number of potential biomarkers that may constitute the basis for diagnosis.6–8 In the case of an acutely ill patient, such as those with suspected native septic arthritis or full blown acute PJI, joint puncture must be performed as soon as possible. Blood cultures should also be taken before surgery or even starting antibiotic treatment to increase the chance of obtaining causative organisms.9

Suspected chronic PJI though is most often a quite different scenario. Although some suspicious clinical features such as a history of prosthetic joint infection (PJI), early loosening, previous wound healing disorder or elevated C-reactive protein increase the probability of infection, virtually every painful prosthesis should be investigated to rule out infection.6,10,11 In some instances, a draining wound or sinus tract will make the diagnosis of PJI obvious. In these cases, superficial swab cultures are tempting but they should be interpreted cautiously. Although they may be informative (especially if virulent microorganisms such as Gram-negative bacilli or S. aureus are isolated in patients with suspected acute postoperative PJI) they are mostly unreliable in chronic draining sinus and often are positive for colonizing/contaminating bacteria.12,13

Joint puncture must ideally be undertaken after a minimum two-week antibiotic-free period. Otherwise, diagnostic accuracy will be significantly compromised.14 It should also be performed under strict aseptic conditions in order to minimize the risk of iatrogenic contamination and to allow for further procedures (Fig. 1). If an insufficient amount of liquid is found, saline injection and subsequent reaspiration may be a useful technique.15 Naturally, if such a technique is used microbiology is the only feasible investigation. Although a positive Gram stain result may still be considered useful information, it should never be used to rule out infection as it has been shown to have very low sensitivity.16,17 Despite its lack of sensitivity and even some disagreement between preoperative and intraoperative bacteriological samples, traditional cultures of aspirated joint fluid remain an important feature of preoperative diagnosis.18,19 Specificity is quite high but even a positive culture must be interpreted cautiously with other diagnostic tests.6,20 It is important to highlight that low-virulence microorganisms such as Cutibacterium acnes or coagulase-negative staphylococci are often contaminants.21

In addition to arthrocentesis, preoperative fluoroscopic-guided biopsies of periprosthetic synovial tissue22 or bone–prosthesis interface membrane23 may also be helpful in establishing a diagnosis in doubtful cases (Fig. 1).

Given that most such infections will require some kind of surgical intervention, deep tissue sampling is usually considered to be the gold standard. Considering the etiopathology of implant-related infections, multiple surgical samples should be collected. Multiple sampling increases the chance of growing a pathogen (i.e. sensitivity) and

Fig. 1 Clinical aspect of fluoroscopic-guided hip arthrocentesis (A) and percutaneous biopsies (B).
it also allows more correct interpretation in cases where a low-virulence microorganism is found. Specificity is increased by interpreting the number of samples in which such species is grown. If more than one sample grows the same indistinguishable organism it is likely that it is indeed a pathogen and should not be dismissed as contaminant.\textsuperscript{6,24} Tissue sampling should be obtained from different sites within the joint and special consideration should be paid to obtaining samples from the bone–implant interface.\textsuperscript{25–27} To reduce the risk of cross contamination, they must be taken with different set of instruments and sent to the laboratory separately.

Lastly, biofilm dislodging techniques such as sonication are extremely helpful, especially when dealing with chronic low-grade implant-related infections.\textsuperscript{25,28,29} It is important to stress that sonication should not be considered an alternative to multiple tissue sampling but rather an add-on test especially useful in patients who have undergone previous antibiotic therapy.\textsuperscript{30–32}

### Osteomyelitis and fracture-related infection

Whether it starts off as hematogenous or fracture-related infection, chronic osteomyelitis shares a lot of clinical features. Diagnosis may be evident if a sinus tract, wound breakdown to bone or implant or pus are present.\textsuperscript{33} Nonetheless, even in this scenario, accurate identification of the microorganism(s) responsible for infection is critical to ensure correct antibiotic therapy.

Again, superficial swab cultures, tempting as they may be, should not be considered adequate sampling as they have consistently been shown not to correlate adequately with deep biopsy or tissue specimens.\textsuperscript{34} Sinus tract cultures are also traditionally considered unreliable in predicting final microbiological results.\textsuperscript{35–37} Nevertheless, it has been shown that two consecutive sinus tract cultures with bone contact at different times may be informative in monomicrobial osteomyelitis if they offer identical results.\textsuperscript{38} Unlike other clinical settings that will be discussed ahead, preoperative percutaneous biopsies are rarely of interest in chronic osteomyelitis as diagnostic yield as well as concordance with definitive cultures results are low.\textsuperscript{39,40}

As such, multiple surgical samples are indisputably the best way to achieve reliable identification of the microorganism(s) involved in osteomyelitis and fracture-related infection (fRI). Bacteria that may be considered skin commensals (such as coagulase-negative staphylococci, \emph{Corynebacterium} or \emph{Propionibacteriaceae}) account for a significant proportion of cases, especially in fractures and should never be dismissed without adequate consideration.\textsuperscript{41} As with PJI, at least five samples should be collected during surgery both to increase sensitivity and help interpret positivity with such low-virulent microorganisms. Samples should be collected in a structured process, with separate instruments for each sample, avoiding touching the patient’s skin with the sample or instrument.\textsuperscript{33,42} Tissue samples should be obtained from infection-suspected deep tissues and not superficial tissue or fluid. Bone samples, especially sequesters or loose infected bone fragments, should always be collected in chronic osteomyelitis.\textsuperscript{43,44}

Fracture-related infections are additionally problematic due to the presence of biofilm on the surface of implants. In these cases, tissue adjacent to the fracture, preferably from the implant–bone interface, should be favoured. Whenever possible, sonication of removed implants may also be performed as an adjunct to multiple tissue sampling.\textsuperscript{32,45}

### Spinal infection

There are mostly two different types of spinal infections, primary hematogenous infections and surgical site infections. There are substantial differences regarding their pathophysiology, and they should be considered when deciding where and how to collect microbiology samples.

Hematogenous infections such as spondylodiscitis, facet joint septic arthritis or epidural abscess arise from hematogenous seeding of the axial skeleton from remote infected foci.\textsuperscript{46} As such, blood cultures should routinely be collected. Their effectiveness in identifying the causative microorganism averages 58% (range, 30–78%).\textsuperscript{47} Other clinically obvious foci such as urinary tract or abscesses should also be investigated.\textsuperscript{46}

Some patients, presenting with symptomatic cord compression with neurologic deficits, will require urgent surgery followed by empirical broad-spectrum antibiotics.\textsuperscript{46} In these cases, open surgical biopsies during the procedure should naturally be collected as they offer the best diagnostic yield.\textsuperscript{48,49} However, most cases will not require urgent surgery. Except in critically ill patients with signs of sepsis, empiric antibiotics should be withheld until every effort has been made to collect adequate samples to establish a microbiological diagnosis as it will have a significant impact.

In patients with negative or unclear blood cultures results (ex. a single set growing coagulase-negative staphylococci) a computed tomography (CT)-guided biopsy should be scheduled as soon as possible. It is noteworthy that this technique is far from being a panacea, offering positive results in no more than 30–60% of cases.\textsuperscript{49–55} Positivity is higher in cases where imaging is consistent with infection\textsuperscript{52,53} and those with higher C-reactive protein. When the initial biopsy is negative, a second attempt may be performed although its real worth is unclear with results in the literature ranging from 0–60%.\textsuperscript{51,55,56}
Diabetic foot infection

Diabetic foot osteomyelitis (DFO) is usually caused by contiguous spread from an infected foot ulcer. Foot ulceration is the most frequently recognized complication of long-standing diabetes and typically originates from repeated microtrauma due to a combination of foot deformities, peripheral neuropathy and/or peripheral artery disease. Patients with DFO have worse outcomes, more surgeries and amputations, longer hospitalizations, and higher rates of recurrent infection and readmission for infection than patients with soft tissue infection. Naturally, clear identification of the infecting microorganism(s) is required for optimal treatment guidance.

Wound swabs are minimally invasive, easy to perform and widely employed in clinical practice, but their findings need to be appreciated and valued according to the specific clinical context. In clearly superficial ulcerate, when the swab is the only sampling method available, the Levine swab technique, where a swab is rotated over a 1 cm² area for five seconds with sufficient pressure to extract fluid both from the wound surface biofilm and from underlying tissues, may be a valid alternative to collect a superficial sample. Nevertheless, it is critical to acknowledge that organisms cultured from superficial swabs usually are not reliable for predicting the pathogens responsible for deeper infection and are also more prone to contamination.

When infection runs even deeper and there is clinical or radiographic evidence of osteomyelitis, deep tissue needle aspiration, deep wound swabs taken through the discharging ulcer probing to the bone or even tissue biopsies have also been suggested as alternative methods. However, several studies have shown poor correlation between cultures obtained by soft tissue and bone sampling, suggesting that soft tissue samples are inadequate to guide DFO antibiotic treatment. As such, percutaneous bone biopsies have emerged as the best sampling alternative in patients not undergoing surgical debridement and have been widely recommended by major medical associations in the field, especially in patients at risk for antibiotic-resistant microorganism(s) and with unclear soft tissue culture results. Biopsies should be performed under fluoroscopic or CT guidance and the needle should traverse uninvolved skin. This is a technically simple procedure that can be easily performed in an outpatient setting without significant complications (Fig. 2).

Sample transport and laboratory processing

A first guiding principle that should always be recognized is that samples collected for microbiological investigation must be sent for laboratory processing as quickly as possible. The longer it takes for bacteria to reach the culture media, the less likely it will be that they are actually grown. A second critical point is that samples should be inserted into sterile transport containers immediately after being obtained. This is especially relevant during surgery. It is not an uncommon error to place samples on the table for the duration of the surgical procedure. It has been clearly shown that a significant proportion of false positives may arise in this manner.

still unclear why the diagnostic yield is so low in comparison to other specimen acquisition methods. Unlike other contexts in orthopaedics, it is not clear that bone tissue samples are advantageous over soft tissue sampling with conflicting results in the literature.

Postoperative surgical site infections are, nowadays, the most common form of spinal infection. They occur more commonly after spinal instrumentation compared to simple lumbar decompression or microdiscectomy, and clinical presentation varies accordingly. Patients presenting with obvious signs of infection such as wound dehiscence and purulent drainage will often require wound exploration and surgical debridement during which unhealthy looking, preferably deep, tissues should be collected for microbiological diagnosis. As previously discussed, antibiotics should be withheld until sample collection except in patients systemically ill or septic. In these cases, blood cultures should be taken before broad-spectrum antibiotics are initiated.

Most patients will present a much more subtle clinical picture mainly characterized by excessive pain around one month after the procedure. Although percutaneous CT-guided biopsy may be informative, especially in postprocedural diskitis, it is seldom required after spinal instrumentation.

Unlike hematogenous infections the vast majority of implant-associated infections will require some form of surgery. Therefore, open surgical sampling is preferred. Spinal implant-related infection treatment follows similar principles as for other implants such as prosthesis. Early infections may be addressed without hardware removal but late infections often require removal of implants. In such cases, culture of removed hardware may be extremely useful especially if biofilm-dislodging techniques such as sonication are used. It is becoming increasingly clear that occult infections are present in a significant proportion of presumably aseptic failures requiring revision surgery. Systematic sample collection for microbiology investigation is therefore recommended in every revision spinal surgery especially in the context of failed fusion.

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Traditionally, synovial fluid recovered from suspicious infected joints is sent to the laboratory in simple sterile vials. Only after reaching the laboratory would the fluid be inoculated onto culture media plates. In addition to reducing the time between harvest and processing, inoculation of synovial fluid directly into blood culture bottles offers a number of advantages. Firstly, it is a highly sensitive method which is especially important in scenarios where a small amount of fluid is recovered and/or there are a presumably low number of viable bacteria present. Secondly, it allows for identification of a broader spectrum of pathogens including slow-growing bacteria without requiring culturing several different and enriched culture media. Finally, automatic systems associated with processing blood culture bottles allow quicker bacterial identification with minimal human errors. The advantages of blood culture bottles have consistently been proven either for the diagnosis of true native septic arthritis or even prosthetic joint infections. This method is also being used to study other fluids such as sonication fluid originating from implant-related orthopaedic infections with favourable results.

As previously discussed, multiple tissue samples are often recommended, but processing all these samples separately must be carried out fastidiously and is very time-consuming. This is especially true in the demanding setting of prosthetic joint and other implant-related infections where its biofilm, often polymicrobial, nature recommends the routine use of an assortment of media suitable for recovery of fastidious, slow-growing, anaerobic and sublethally damaged bacteria such as Chocolate agar, MacConkey agar, Thioglycollate broth, etc. Moreover, it has been shown that this process should be extended for up to 14 days. Given its relative ease and simplicity, the use of blood culture bottles to process samples has also gained considerable popularity for processing bone and soft tissue samples. The main difference is that beads and vigorous shaking must be used to disrupt tissue and release bacteria. This can be done by manually adding sterile glass (Ballo-tini) beads using an aseptic technique in a safety cabinet or by using specific commercially available vials (Fig. 3). Once the sample is ‘liquified’, aliquots are inoculated into culture bottles. Since anaerobic bacteria are significant pathogens...
in implant-related infections, it is important to use both aerobic and anaerobic vials in such circumstances. The use of automated blood culture bottles systems to process tissue samples has consistently been shown to result in improved diagnostic accuracy. In addition, time to positivity from surgery to results is significantly shorter, which translates into shorter broad-spectrum antibiotics periods.

As has already been discussed, sonication of removed implants may be a useful adjunct. It must be noted that it requires simple yet specific equipment that is not widely available in most laboratories. Once a decision is made to use sonication, it is important to point out that implants should be placed into sterile solid containers with airtight seals, as it has been shown that plastic bags are prone to contamination and therefore lack of specificity. The implant should be (at least for the most part) covered in saline or Ringer’s solution and subsequent laboratory processing follows a sequential vortexing–sonication–vortexing validated protocol before aliquots of sonication fluid are inoculated into selected culture media (Fig. 4).

**Conclusion and future developments**

Infections are defined by pathologic invasion and growth of germs in the human body. Adequate sampling and microbiological identification is critical. The specific pathophysiology of bone infections, especially the presence of biofilm in implants, is such that it is not uncommon for classic microbiological investigation to offer suboptimal results. As such, there is a growing body of knowledge allowing for definition of infection even without classic microbiologic confirmation. Highly sensitive molecular diagnostics techniques will certainly play a role in the future in these so-called culture-negative infections. They are already able to identify pathogens and even to determine the presence of certain antibiotic resistance genes. Despite their early promise, they are neither widely studied nor readily available in most laboratories.

For the time being, treating physicians must rely on adequate sampling to offer the best chance to identify the infecting pathogen(s). Ideally, decisions regarding complex bone and joint infections will be made in a multidisciplinary team setting. Knowledge about how and when the sample was collected and processed is critical for accurate interpretation of the available information. The orthopaedic surgeon is often the one responsible for obtaining specimens and must therefore be knowledgeable about the nuances around this topic.

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