

## QUESTION 2: What methods can be utilized to increase the diagnostic yield of microbiological culture in surgical site infection/periprosthetic joint infection (SSI/PJI)?

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**RECOMMENDATION:** At least four intraoperative cultures should be obtained to increase the diagnostic yield. There is limited evidence to suggest that cultures from the synovium, synovial fluid or tissue in contact with prosthesis may be more likely to identify a pathogen. The samples should be inoculated in blood culture bottles and the addition of enriched media (such as a chocolate agar plate and Schaedler broth) or bead mill processing broth may also augment yield.

**LEVEL OF EVIDENCE:** Moderate

**DELEGATE VOTE:** Agree: 86%, Disagree: 9%, Abstain: 5% (Super Majority, Strong Consensus)

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### RATIONALE

Identifying an organism from microbiological culture is critical for both the diagnosis and treatment of SSI and PJI [1–3]. Two positive cultures from the same joint identifying the same organism by tissue or fluid remains as one of the major criteria for the diagnosis of PJI in total joint arthroplasty (TJA). This qualifies as a “major” criterion in both 2013 and 2018 definitions of PJI [2,4]. However, in 7 to 35% [5–9] of patients, no organisms can be isolated despite meeting other criteria for infection, which defines “culture-negative” PJI patients [3]. In general, and particularly for this cohort of patients, optimizing culture yield can help determine type of surgical procedure, antibiotic therapy and likelihood of treatment success.

Methods of optimizing culture growth have been divided into preoperative, intraoperative and postoperative measures. With regard to preoperative measures, the American Academy of Orthopaedic Surgeons’ Clinical Practice Guidelines (AAOS CPG) recommends aspirating a joint for culture at least two weeks following the last administration of antibiotics (moderate recommendation) [1]. If growth is unsuccessful initially, a repeat aspirate is recommended (consensus recommendation for knee, moderate for hip). Finally, if the diagnosis of PJI is suspected but not confirmed, holding antibiotic treatment is recommended in an attempt to identify an organism pre- or intraoperatively (strong recommendation) [1]. Intraoperative measures for optimizing culture growth include obtaining multiple cultures prior to irrigation and obtaining cultures from representative areas (i.e., intramedullary, implant interface). The samples for culture should also be obtained using a clean instrument and transferred immediately to the culture bottle for transport. The culture samples obtained should also be transported to the laboratory as soon as collection is complete.

Postoperative measures include choice of growth medium, bead mill processing, timely delivery to and processing by the laboratory, use of sonication and culture duration. The scope of this question will address the following: What is the right number of intraoperative cultures, what type of cultures should be obtained, which areas should be sampled, does bead mill processing increase yield and what is the best growth medium. The remainder of the measures to optimize growth are covered by other International Consensus Meeting (ICM) questions.

The AAOS CPG recommends that multiple cultures be obtained at the time of surgery (strong recommendation), but no number was provided. The 2013 ICM recommended that three to five cultures be taken in the setting of suspected or uncertain PJI (strong consensus) [10]. Previous studies recommended that five cultures be obtained [11–13] but Atkins et al. were the first to evaluate this prospectively and perform statistical analysis. They examined cultures grown from 297 revision arthroplasties and found that 5 to 6 cultures increased the likelihood of diagnosis [14]. In 2016, Bémer et al. published a prospective, multicenter study that found using four culture samples on three different growth media was a highly reliable and cost-saving approach to PJI diagnosis [15]. Gandhi et al. corroborated these results by examining 74 PJI patients meeting Musculoskeletal Infection Society (MSIS) criteria [16]. They found that the optimal number of cultures needed to yield a positive test result was four (specificity = 0.61 and sensitivity = 0.63) and concluded that increasing the number of samples increased sensitivity but reduced specificity [16]. Finally, Peel et al. also determined that a minimum of four cultures were optimal to achieve growth with conventional means but a minimum of only three cultures were required when using blood culture bottles [17]. Some authors have advocated up to 10 cultures in the setting of prior antibiotic use and less virulent organisms [18] but these situations may be ideal for the use of emerging technologies such as next generation sequencing [19].

With regard to how samples should be obtained, studies are mixed on whether synovial fluid culture is superior to tissue culture [15,16,20,21]. However, both are often obtained simultaneously in clinical practice and in combination increase the sensitivity for diagnosis [20]. Multiple studies have demonstrated that swabs are not a reliable culture method intraoperatively [7,22]. Due to their high rate of false-negative and false-positives [23], their use is strongly recommended against by the 2013 ICM [10]. It is often stated that cultures should be removed sharply with a scalpel, handled with clean instruments and placed directly into the sterile container. However, to the authors’ knowledge, no studies have investigated the role of the technique to obtain the samples and culture yield.

It is often recommended that cultures be obtained from the intramedullary canal and bone-implant interface [24]. However, Gandhi et al. investigated the role of a “best culture.” This is a practice used to identify a promising specimen from anywhere in the infected joint that should undergo additional testing (i.e., fungal and mycobacterial) beyond routine aerobic and anaerobic cultures [16]. Despite being a visually appealing specimen, this “best culture” practice did not increase the likelihood of growth [16]. In addition, Bémer et al. in a multicenter prospective study found the highest rates of culture positivity from synovial fluid 91.7%, followed by tissue in contact with implant material (91.5%) whereas bone samples had the lowest rates of positive cultures (76.6-87.1%) [15].

Once a culture is obtained, but prior to inoculation, a process known as bead mill processing may also be used. The process involves placing tissue specimens into sterile vials, adding a small amount of sterile water and beads (glass or metal) and adding mechanized agitation (bead mill) [15,25]. One study has reported improvements in PJI diagnosis when using this technique [25]. Another prospective, multicenter study utilized this method and also found higher rates of bacteriologically documented PJI than reported previously in the literature [15].

The use of alternate culture media has also been described to optimize culture growth. Hughes et al. reviewed 805 synovial fluid samples from patients suspected of having septic arthritis [26]. The culture results obtained with a blood culture bottle were compared to those obtained by a conventional agar plate method. The blood culture method identified significantly more pathogens and fewer contaminants compared to the conventional method [26]. Similarly, Font-Vizcarra et al. retrospectively reviewed 87 cases of PJI in 2010 [7]. They compared culture growth of synovial fluid inoculated in blood culture bottles to periprosthetic tissue and swab samples in standard media. Not only did the synovial fluid in blood culture

bottles have a higher rate of positivity, this method also had higher sensitivity, specificity, and positive and negative predictive values for diagnosis of PJI when compared with standard tissue and swab samples [7]. Subsequent PJI studies have also demonstrated that cultures of periprosthetic tissue in blood culture bottles increases culture yield compared to swabs [27], standard agar/broth [28,29] and is similar in sensitivity to sonication [30].

Finally, aside from using blood culture bottles, enriched or organism specific media has also been reported. When suspecting a fungal, zoonotic bacteria, mycobacterium or other unusual microorganisms, routine bacterial and anaerobic cultures will often fail to yield the pathogens [31]. The laboratory should be alerted when these organisms are suspected to avoid accidental exposure and the right media can be chosen such as brain-heart infusion, trypticase soy broth and chocolate agars [31]. Bémer et al. investigated the question of what is the best growth media and found that the most efficient means to identify PJI per their definition was obtained with a combination of three different culture media: a blood culture bottle, a chocolate agar plate and Schaedler broth [15]. The authors also reported that the chocolate agar plate was more sensitive than the anaerobic agar plate, particularly for the anaerobe *C. acnes* [15].

In conclusion, there is evidence to support the use of blood culture bottles, obtaining at least four intraoperative cultures (including synovial fluid and periprosthetic tissue), bead mill processing and enriched media to increase diagnostic yield of microbiological culture in SSI/PJI. Of these, the most studied methods include the ideal culture number and use of blood culture bottles (moderate evidence). The remainder of the interventions listed currently have limited evidence.

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