G71: What are the optimal sampling and processing techniques for cultures obtained during any revision surgery?

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Response/Recommendation: Four to six samples should be submitted to the microbiology laboratory and include at least three periprosthetic tissue samples. Synovial fluid and implant sonication fluid can be sent, if available. Samples should be mechanically homogenized to maximize bacterial extraction and minimize contamination. Prolonged incubation in monitored blood-culture vials increases the sensitivity of culture and should be substituted to classical broth enrichment when possible.

Level of Evidence: Moderate

Delegate Vote:

Rationale:

The diagnosis of PJI remains difficult and composite clinical scores aggregating clinical, microbiological, biological and pathological criteria are frequently used as gold standard.

The microbiological documentation of PJI allows not only the unambiguous recognition of infection but allows optimal care through personalized antimicrobial treatment. The culture of the same microorganism from multiple samples gathered in the course of the revision surgery is commonly required for its consideration as a causative agent of infection.

The sensitivity of periprosthetic tissue culture to diagnose PJI with full microbiological documentation has been reported to range from 57% [1] to 92% [2] . Major factors governing these performance discrepancies arise at every stage of the analytical process: biopsy collection and transport, biopsy homogenization, choice of media and incubation atmosphere, automated monitoring, implant analysis and synovial fluid culture. We acknowledge that culture negative implant associated infections exist. Other factors such as formation of biofilm on the implant, prior antibiotic therapy, or infection with fastidious organisms contribute to culture negative infections [3, 4]. In these circumstances molecular biology approaches, PCR based or metagenomic, may play a role in identification of the infective organism [5, 6].

Although no specific anatomical sampling sites has shown to provide higher diagnostic yields and should be targeted at macroscopically abnormal tissues, taking samples from deep bone samples [7] and the intramedullary canal [8, 9] is considered best practice. The use of swabs is discouraged due to poor performance [10]. Based on recommendations of Infectious Disease Society of North America (IDSA) and others, consideration should be given to refrigeration of samples, if processing of the samples is expected to be more than two hours from the time of colletion [11-13]. Although definitve data supporting the latter recommendation is lacking, *in vitro* evidence supports this notion [14]. Additional clinical evidence can be derived from the benefit of the addition of anaerobic media during the transport of samples to the laboratory, enhancing diagnostic performance and completeness of microbiological documentation [15, 16]

although a study failed to provide evidence in this regard [17]. Kaschner et al. showed that the benefit of thioglycolate broth was not related to the temperature of incubation, suggesting that the pH buffer, redox potential, cationic ions or osmolality of the medium was providing the performance enhancement. The use of double wrapped sterile packaging cleared for operating theaters and mechanized processing has decreased contamination rates thanks to the limitation of the samples handling steps [18].

The microbiological analysis requires the exposure of the pathogens to the culture medium to allow its growth and detection. Several methods have been reported to release bacteria from solid samples. Scalpel dissection of the samples or mortar and pestle grinding in broth were the most common techniques. Vortexes can be used with broth or saline with glass beads [19]. Mechanical homogenization has been performed with different instruments, such as a paddle blender [20], a beadmill with glass beads [18], beadmill with steel beads [21, 22], beadmill with ceramic beads [23], dispersion with single use ultra-turrax device [24]. The use of these mechanized disruption methods have allowed a substantial increase in sensitivity, and allowed the use of blood culture systems to monitor and enhance bacterial culture.

The number of samples and number of samples positive with the same micro-organism has evolved as the methods grew more standardized. As early as 1981, Kamme proposed to culture 5 periprosthetic tissue biopsies and claimed that a microorganism growing on 2 or more samples was indicative of infection. Five to 6 samples with positive growth from 3 or more samples was then broadly used [25]. The less stringent breakpoint of growth of 2 or more identical isolates became consensual [25], IDSA recognizing PJI with a single sample growing with a virulent species [26]. Using current microbiological methods employing mechanized sample processing and blood culture vials for monitoring of the samples, 3 to 4 periprosthetic tissue samples now appear to be optimal for sensitivity and specificity [21, 27-29].

Synovial fluid has proven to be a reliable sample for culture as well as cytological and biochemical analysis. The immediate seeding of blood-culture vials with synovial fluid has led to the improvement of culture sensitivity [30]. A sample should nonetheless be sent for cytological analysis on a vial with anticoagulants.

The retrieval of biofilms from implants using ultrasound treatment (sonication) has been initially used for immunofluorescence [31] but has been popularized by Trampuz et al. for culture [32]. The use of Dithiothreitol to dislodge biofilm bacteria from implants has been advocated with contradictory results [33-35] when compared to culture or sonication. An independent meta-analysis [36] did not find a significant difference between both methods and further studies would be warranted to compare the performance between Dithiothreitol and sonication. An extensive literature has described the increased sensitivity of implant sonication compared to tissue culture [37], but most recent publications using mechanized disruption of tissue samples achieve comparable results [38]. The combination of implant-retrieved biofilm and periprosthetic tissue cultures seems to further increase diagnostic yield [1, 20].

Due to the diversity of organisms present in periprosthetic joint infections, aerobic, anaerobic and CO_2 atmospheres should be used. Solid media in aerobic, anaerobic and 5% CO_2 should be used for the culture of periprosthetic tissue samples and synovial fluid. It is unnecessary to routinely use fungal and mycobacterial growth media [39, 40] and should only be performed in high-risk cases or in secondary documentation of a culture negative PJI. Broth enrichment significantly enhances diagnostic yield over solid media [21] although discrepant studies [41,

42] emphasize the benefit of larger inocula and minimizing sample handling steps with broth enrichment. The use of blood culture vials an as enrichment broth has shown a significant improvement in sensitivity [2, 20, 21, 43-45]. There is clear evidence that both aerobic and anaerobic media should be used [43], and the use of resin or charcoal-free anaerobic blood culture media are required for the culture of Gram positive anaerobes, *Cutibacterium acnes* in particular requires the combined use of solid and liquid culture media [46, 47]. The recommended incubation durations for aerobic media have shown that there is no benefit to pursuing it beyond 7 days [21, 48], although anaerobe media should be incubated up to 14 days [21, 47, 49]. Using state of the art tissue processing and high-volume inoculation of blood culture bottles, definitive diagnosis can be reached in <48h in a majority of cases [21, 43, 44].

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