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QUESTION 17: What is the optimal time for culture processing of tissue or synovial aspirate samples? How long should routine cultures be kept before declared negative?

RECOMMENDATION: Cultures should be maintained for a period of five to seven days. In cases of suspected periprosthetic joint infection (PJI) with low-virulence organisms or if preoperative cultures have proven to be negative and there is a high clinical suspicion for PJI (culture-negative PJI), the cultures should be maintained from 14 to 21 days.

LEVEL OF EVIDENCE: Moderate

DELEGATE VOTE: Agree: 87%, Disagree: 12%, Abstain: 1% (Super Majority, Strong Consensus)

RATIONALE

It is believed that the majority of common infecting organisms can be isolated within a few days of conventional culture. Additionally, there is currently no reason to extend the culture duration in patients in whom the infecting organism has been isolated preoperatively. Research has focused on the incubation period for samples from patients with suspected PJI, culture negative cases and patients who may be infected with low-virulence organisms, such as *C. acnes* and anaerobes. Unfortunately, there is no consensus on an appropriate culture time, although identifying the responsible infectious agent is critical in PJI [1].

There exists a notion that longer incubation times may increase the possibility of detecting contaminants and thus false positives [2]. However, numerous studies have demonstrated that extending culture time to two weeks significantly increases the culture sensitivity without increasing the risk for the growth of contaminants [1–5]. Currently, there is no evidence determining the cost-effectiveness associated with holding cultures for one week versus two weeks. Besides the matter of cost, it remains critical that cultures are held for an adequate amount of time in an effort to isolate any potential pathogen for even cases that are presumed aseptic [6,7].

Most tissue or synovial cultures are incubated for five days or less [8], however, there are studies underlying the importance of extending this period [1,5,9]. Butler-Wu et al. tried to identify the optimum culture conditions for recovery of *C. acnes* from PJI specimens [5]. They applied 28-day culture incubation to all specimens from 198 revision arthroplasties and found that minimum 13-day culture incubation for both aerobic and anaerobic cultures is necessary for diagnosing *C. acnes*. Incubation beyond this period was non-diagnostic for *C. acnes* isolates. Schaffer et al. proposed that microbiological culture should be held for 14 days to diagnose infection in patients after conducting a large prospective study, in which tissue samples from 284 patients were cultured [1]. Although the median time to diagnosis of a suspected organism was only 4 days, additional organisms causing PJI were grown up to 13 days later, further highlighting the polymicrobrial nature of PJI. Comparing early versus late detected organisms, they demonstrated that the early group was composed of staphylococci, enterococci, etreptococci and enterobacteria. These organisms grew within the first seven days of culture. The late group, growing predominantly from 7 to 14 days, exhibited growth from Propionibacterium species, aerobic gram-positive bacilli and *Peptostreptococcus* species.

Neut et al. evaluated a cohort of 22 patients with suspected septic loosening. They concluded that by prolonging the culture time to 7 days, it increased the detection rate of infectious bacteria from 41% to 64% [4]. Bossard et al. recommended that culture specimens should be kept for at least 10 days to detect *C. acnes* [10]. In their retrospective study examining 70 *C. acnes* infections, they found that in reducing the culture period to 7 days, diagnosis of PJI would have been missed in 21.4% of the cases. Despite their recommendation of a 10-day culture period, 6% of these *C. acnes* infections were identified outside the 10-day culture period. The similar conclusion about *C. acnes* was made by Framgiamore et al. who showed that 14% of the culture-positive cases were detected after day 7 in their review of 46 cases [11].

Additionally, there is literature proposing that a prolonged period of incubation (up to 21 days) is required to minimize the culture-negative PJI rate [12]. Parvizi et al. proposed that cultures should be kept for at least 14 days and if no microorganism is isolated, an additional 7 days of incubation may be required. An additional seven days of incubation may allow for the isolation of slow-growing organisms such as Mycobacterium species and fungi [12]. Utilizing a prolonged incubation period may be useful for cases where no organism is identified preoperatively.

Novel techniques have emerged to increase detection rates and minimize the culture period required in the diagnosis of PJI. In a prospective laboratory study over a seven-month period, tissue samples were taken from patients with suspected PJI [13]. All samples were cultured for 14 days, using a BD BACTEC™ instrumented blood culture system. All but 1 out of the 66 culture-positive cases of PJI was detected within 3 days of incubation. The use of blood culture bottles was valuable for increasing the diagnostic sensitivity for PJI. A more recent study evaluated culture time for anaerobes and proposed a modern laboratory procedure that could improve detection and shorten culture time [14]. They showed that all pathogens could be identified within six days using a highly sensitive media (supplemented liver thioglycollate broth) and with direct identification by matrix-assisted laser desorption/ionization (MALDI-TOF).

To date, there are numerous techniques and methodologies utilized in conventional culture. Current literature suggests that cultures should be kept and processed on the basis of the infecting organism. Cultures should be processed and kept for at least five days. In cases of suspected PJI with low virulence organisms or if preoperative cultures have proven to be negative and there is a high clinical suspicion for PJI, cultures should be maintained for at least 14 to 21 days.

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