SH56. What is the optimal culture technique (e.g. culture medium, days of cultivation) in evaluating patients for infections of the shoulder?

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Recommendation/Response:

Current evidence suggests that culture of tissue samples for the diagnosis of shoulder PJI is best performed using liquid culture in both aerobic and anaerobic conditions. Blood culture bottles on a semi-automated platform offers the most efficient liquid culture technique. Diagnostic accuracy may be improved by using two or more different culture media. Optimal duration of incubation varies with the choice of culture medium. Prolonging duration of incubation beyond 10 days in blood culture bottles is likely to yield minimal additional benefit. The duration of time to detection is dependent upon the specific bacterial organism, as well as the bacterial load. The results of prolonged culture need to be carefully interpreted.

Strength of the recommendation: Moderate

Rationale:

Shoulder PJI is a common indication for revision arthroplasty surgery (Fink & Sevelda, 2017). Determining and confirming the diagnosis can be difficult, especially in chronic cases with low virulence organisms. This requires identification of the infecting organism so that appropriate treatment can be rendered. *Staphylococcus species* and *Cutibacterium acnes (*formally *Propionibacterium acnes)* are the most commonly isolated bacteria. While culture techniques and interpretation of culture results for the former are well established, it was not until relatively recently that *C. acnes*, a ubiquitous commensal bacteria in human skin, especially around the shoulder, was clearly recognized as a pathogen in shoulder PJI. Nevertheless, due to the fact that *C. acnes* is so prevalent, distinguishing between the presence in culture as a contaminant versus actual pathogen can also be challenging. In addition, studies report positive *C. acnes* cultures of negative control specimens. Diagnosis is also complicated by the fact that *C acnes* PJI is often associated with few local or systemic signs of inflammation and is slow growing in laboratory culture. Defining the optimal culture technique in the evaluation of shoulder PJI is therefore important, especially in cases of chronic infections. However, even if this is achieved, culture is likely to yield a proportion of false positive results and clinical interpretation can be difficult. Further development, utilization, and investigation of other diagnostic tools is needed and will hopefully improve diagnostic accuracy in the future.

There are three main variables relating to culture conditions for the diagnosis of shoulder PJI.

Duration of culture

In order to optimize detection of bacterial organisms, including *C acnes*, in shoulder PJI, most authors advise prolonged culture incubation. A number of studies have investigated this issue and while the ideal culture duration has yet to be firmly established, it is clear that cultures should be continued for at least 7 days. While too short an incubation time may limit the sensitivity, too long

an incubation time may result in false positive cultures, with the isolation of non-diagnostic or contaminant organisms, thereby limiting the specificity. Additionally, there are other factors such as bacterial type and burden that can affect the results of aerobic and anaerobic bacterial tissue culture.

Many authors advise a minimum incubation period of 14 days (Parvizi & Gehrke, 2014), (Fink, et al., 2008), (Ince, et al., 2004), (Nodzo, et al., 2017) and some advise at least 21 days (Pottinger, Butler-Wu, & Neradilek, 2012), (Fink & Sevelda, 2017).

Schafer and colleagues (Schafer, et al., 2008) reviewed tissue culture and histological specimens from 284 cases of hip (145) and knee (139) revision with suspected PJI. Infection was diagnosed in 110 patients. After 7 days (the longest incubation period most frequently reported), the detection rate via culture was 73.6%. The median time to diagnosis was 4 days. However, bacteria associated with infection were found up to 13 days. Early detected bacteria (e.g. *staphylococci, enterococci, streptococci, and Enterobacteriaceae*) were mostly during the first week, whereas late detected bacteria (e.g., *Propionibacterium* species,
aerobic gram-positive bacilli, and *Peptostreptococcus* species) were detected during the second week. For early and late identified bacteria there was a correlation between the number of culture-positive tissue samples and positive results of histologic analysis. Contaminants were detected significantly later than were infecting organisms although the median time to detection was only 7 days. Thus, as many as 52% of the contaminating strains emerged during the first week of culture. This contradicts the common thought that contaminants dramatically increase with prolonged cultivation. They recommended an incubation period of up to 14 days based especially on late recovery of aerobic Gram-positive rods and *Cutibacterium* species.

Butler-Wu et al (Butler-Wu, Burns, & Pottinger, 2011) studied 198 revision joint arthroplasties using a 28 day culture incubation protocol. 87 cases (83% were from shoulders) had at least 1 positive bacterial culture. 42 cases were considered infected. *P. acnes* was identified in 23 cases (4 polymicrobial). *P. acnes* was also the organism most commonly isolated from non-infected cases. All *P. acnes*- were positive for growth by 13 days in the infected cases. Furthermore, incubation beyond this was associated with increasing recovery of non diagnostic *P. acnes*: in 21.7% of nondiagnostic cases cultures became positive after 13 days. These data suggested that for true infection there is a reduced time to positivity compared with nondiagnostic cases. They did not find a specific culture medium that was superior for the detection of *P. acnes* PJI. Positive cultures for *P. acnes* from sheep blood agar were exclusively associated with true PJI. However, all specimens with *P. acnes* recovered from sheep blood agar also had *P. acnes* recovered from additional culture media. Interestingly, a diagnosis of *P. acnes* PJI would have been missed in 29.4% of patients had extended culture incubation only been employed for the anaerobic culture media, demonstrating the importance of applying an extended culture incubation period to both aerobic and anaerobic culture media.

Considering the 28 day culture period, specimens from true infections with *P. acnes* were 6.3 times more likely to have 2 media positive for growth than noninfected cases *P. acnes* events ($P = 0.002$). If a 14-day culture incubation period had been considered, *P. acnes* infected events were 9 times more likely to have 2 media positive for growth with *P. acnes*. Lastly, the presence of 3 or more positive cultures with *P. acnes* was exclusively associated with infection. Their results support the

need for a minimum culture incubation period of 13 days to be applied to both aerobic and anaerobic culture media for all periprosthetic specimens.

Peel et al (Peel, et al., 2016) compared the sensitivity and specificity of culturing PJI tissues in blood culture bottles (BCB) and standard agar media. They included 117 (17 shoulder) cases of PJI as defined by the Infectious Diseases Society of America criteria. Inoculation of tissues into BCBs was associated with a 47% improvement in sensitivity compared to the sensitivity of conventional agar and broth cultures (92.1 versus 62.6%, respectively); this magnitude of change was similar when IDSA criteria were applied (60.7 versus 44.4%, respectively; P=0.003). The time to microorganism detection was shorter with BCBs than with standard media (P< 0.0001), with aerobic and anaerobic BCBs yielding positive results within a median of 21 and 23 h, respectively. The median time to positivity using blood culture bottles was less than 24 hours and there was no additional yield from incubation of aerobic bottles beyond 7 days. Anaerobic bottles detected pathogen growth more rapidly than agar or thioglycolate broth but extending incubation beyond 7 days yielded a diagnosis of PJI in five additional subjects who fulfilled the IDSA diagnostic criteria.

Minassian and colleagues (Minassian, et al., 2014) prospectively analyzed 332 revision arthroplasty patients whose surgical samples were processed using both blood culture bottles and conventional media and cultured for 14 days. 79 met criteria for PJI of which 66 were culture positive Amongst the 66 who had microbiologically confirmed PJI, 65 cases were identified as culture positive within 3 days and one at day eight. Based on their results they concluded that prolonged culture incubation of 2 weeks is unnecessary when using the BACTECTM culture methods.

McCarroll and co-workers (McCarroll et al., 2021) performed a prospective study of 95 patients undergoing primary open or arthroscopic shoulder surgery without a history of prior shoulder surgery or injection. They obtained superficial tissue, tissue culture, and ‘‘sterile’’ control Swab for analysis. Cultures were held for 28 days and checked at regular intervals. They reported a 17 percent false positive culture rate in the open group (8/47 cases) and a 10.4 percent false positive rate in the arthroscopic surgery group (5/48 cases). The incidence of positive *C acnes* culture results was 6.4% in the open group, and *C acnes* was not isolated in the arthroscopic group. All positive bacterial culture results were reported within 7 days of collection. They concluded that holding cultures longer than 14 days does not appear to lead to an increase in false positive rates.

The American Shoulder and Elbow Surgeons Periprosthetic Joint Infection Multicenter Group analyzed 12 blinded samples of which 10 were 2 sets of known C. acnes at 5 different dilutions and 2 sets of negative control samples at 11 different institutions. (Hsu, et al 2022) They found that 100 percent of the C acnes samples for the 4 highest dilutions grew and 91 percent of the C acnes samples from the lowest dilution grew. Interestingly, 14 percent of the negative control samples grew C acnes. The mean time to positivity was 4.0 (SD: 1.3) days and all samples showed growth within 7 days. Furthermore, there was a significant inverse relationship between bacterial load and time to positivity (Spearmen r = -0.9, P = .037).

Bokshan and co-workers reported that utilization of a regulated anaerobic incubation chamber significantly reduced the time to positive C. acnes from 6.5 days (range 3–10 days) to 4.9 days (range 2.75–10 days) (mean difference: 1.6 days, 95% confidence interval: 1.06–2.66 days; P=0.002). (Bokshan et al) The time to positive cultures was also significantly shorter in cases with true infections compared to contaminants (5.5 vs 6.8 days, respectively, P=0.003). In addition, in cases with a greater number of positive culture specimens there was a shorter time to culture positivity (Spearman rank =0.58, P ⅟4.007).

Frangimore and co-workers studied culture results from 46 patients who underwent revision shoulder arthroplasty. They reported that the time to *C. acnes* culture growth was significantly shorter ($p = 0.002$) in the probable true-positive culture group (definite or probable infection) compared with the probable contaminant group (median of five days [interquartile range, four to seven days]) compared with nine days [interquartile range, six to twelve days]). Among the thirty-seven cases in the probable true-positive group, no culture result turned positive after eleven days, whereas in the probable contaminant group, cultures turned positive after this time point in 44% (four of nine) of the cases. There were also significantly fewer days to P. acnes culture growth among cases with a higher number of positive cultures ($p = 0.001$) and a higher proportion of positive cultures ($p < 0.001$), regardless of group classification.

In summary, cultures should be held for at least 7 days to avoid false negative culture results, especially when C acne is a potential infectious organism. Holding cultures for up to 14 days appears, in some studies, to increase the yield of positive cultures associated with PJI. False positive or contaminant organism can be identified early and late. Positive culture results beyond 7 days should be carefully interpreted.

Anaerobic and aerobic culture

PJI caused by strictly anaerobic pathogens is rare but mandates careful selection of antimicrobials for optimal therapy. Whilst *C acnes* is an anaerobic organism, many strains are aerotolerant and Butler-Wu and colleagues (Butler-Wu, Burns, & Pottinger, 2011) suggested a significant and clinically important improvement in yield by using aerobic and anaerobic culture conditions. This recommendation is supported by most authors. In addition, some studies support extending the aerobic culture duration .

Choice of culture medium

Conventionally, the laboratory diagnosis of PJI has relied upon culture of tissue specimens on solid media (agar) and in broth cultures. Unless they become visibly turbid, the latter are terminally sub-cultured onto agar to detect any non-visible growth in the broth. This is both time consuming and cumbersome.

Butler-Wu *et al* (Butler-Wu, Burns, & Pottinger, 2011) analyzed the accuracy of *C acnes* PJI diagnosis in 198 revision arthroplasty procedures using four different culture media (blood agar, chocolate agar, Brucella agar and brain-heart infusion (BHI) broth). They found that recovery of *C acnes* from blood agar was exclusively associated with the presence of infection (16 specimens) but all specimens positive for *C acnes* on blood agar were also positive for growth on at least one additional culture medium. BHI yielded the highest number false positive results and Brucella

agar yielded the highest number of true positive results. They reported that specimens from *C acnes*-infected cases were 6.3 times more likely to have 2 media positive for growth than non-diagnostic *C acnes* cases (P 0.002)

Hughes et al (Hughes, et al., 2011) prospectively compared conventional culture media and blood culture medium in 849 separate specimens from 178 patients undergoing arthroplasty revision. They estimated the sensitivity and specificity of blood culture medium to be 87% and 98% respectively. By comparison, the sensitivity of direct plates and cooked meat broth culture were 39% and 83%

Motwani and colleagues (Motwani, Mehta, Aroojis, & Vaidya, 2017) found that, in sixty cases of pediatric septic arthritis caused by any organism, incubation of clinical samples in BACTEC blood culture bottles as compared to conventional agar plates increased the yield from 42% to 71%.

Peel *et al* (Peel, et al., 2016) compared the sensitivity and specificity of culturing PJI tissues in blood cultured bottles and standard agar media. Refer to section on duration of culture for relevant details regarding culture medium.

El Sayad and co-workers investigated the association between *C. acnes* genotype and time to detection in blood culture bottles (Lytic-Ana BCB) seeded with standardized culture inoculum (experimental conditions) and with clinical intraoperative culture specimens (clinical conditions). 72 unique *C. acnes* strains were recovered and analyzed; 24 (33.3%) were considered infectants, and 48 (66.7%) were contaminants. They were distributed among four main clonal complexes (CC). CCs of *C. acnes* in the Lytic-Ana BCBs did not grow at the same rate. In the experimental and clinical conditions, CC53 isolates were detected twice as fast as the other CCs, and 100% of CC53 isolates were detected within 5 days of incubation, compared with 50% or less for the other CCs. Infectant *C. acnes* strains were detected significantly earlier than contaminants in blood culture bottles. However, this difference was no longer present when BCBs were seeded with a standardized culture inoculum. Therefore, the slower growth of contaminant isolates in the clinical setting might be related to a lower bacterial load and thus a lower bacterial inoculum in BCBs. They concluded that the optimal cut-off to distinguish between infectant and contaminant isolates is between five and eight days (with a sensitivity of 80% and a specificity of 61%). Nevertheless, almost 40% of contaminant isolates were detected within 5 days and 20% of infectant isolates detected after 8 days. Their results confirm the need for prolonged incubation of specimens beyond 7 days when liquid-based media are used, even when BCBs are used. Furthermore, inoculation of ODRI clinical specimens in blood culture bottles is not sufficient for optimal detection of C. acnes and requires simultaneous inoculation on anaerobic solid media and possibly other liquid-based media. Up to 35% of isolates grew only on solid media, while 40% of isolates grew only on the Lytic-Ana BCB medium. Inclusion of BCBs most likely improves diagnostic yield, but a definitive switch from standard agar and liquid media to BCB is not advisable. In fact, they stated that BCB can cause selection bias against *C. acne*.

Ellsworth and co-workers retrospectively studied a cohort of patients who were treated for periprosthetic shoulder joint infections. In additional to primary culture media (5% sheep blood agar, chocolate agar, and MacConkey agar), Hemin and vitamin K enriched Brucella blood agar (BBA) plate (Hardy Diagnostics, Santa Maria, CA, USA) was routinely inoculated for all shoulder periprosthetic cultures and incubated anaerobically at 35 C for 10 days. To reduce the chance of

laboratory contamination, if no colonies were identified after 3 full days of incubation, the BBA plate was placed into Anaerobic GasPak bags before being examined again on day 10. Samples were held in standard media for 3 days. 136 cultures from 85 patients (101 tissue cultures) were evaluated. At least 1 culture turned positive in 58 of 136 events (42.6%),
with C acnes being the most frequently isolated.

Blood culture bottles appear to provide enhanced bacterial culture yields in the evaluation of PJI over traditional agar and broth cultures. Nevertheless, the available evidence supports continued use of traditional bacterial culture medium in addition to blood-culture-bottles.

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