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What do positive and negative *Cutibacterium* culture results in periprosthetic shoulder infection mean? A multi-institutional control study

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Background: Deep tissue culture specimens obtained at the time of revision shoulder arthroplasty are commonly positive for *Cutibacterium*. Clinical interpretation of positive cultures can be difficult. This was a multi-institutional study evaluating the accuracy of cultures for *Cutibacterium* using positive control (PC) and negative control (NC) samples. The relationship between time to culture positivity and strength of culture positivity was also studied.

Methods: Eleven different institutions were each sent 12 blinded samples (10 PC and 2 NC samples). The 10 PC samples included 2 sets of 5 different dilutions of a *Cutibacterium* isolate from a failed total shoulder arthroplasty with a probable periprosthetic infection. At each institution, the samples were handled as if they were received from the operating room. Specimen growth, time to culture positivity, and strength of culture positivity (based on semiquantitative assessment) were reported.

Results: A total of 110 PC samples and 22 NC samples were tested. One hundred percent of specimens at the 4 highest dilutions were positive for *Cutibacterium*. At the lowest dilution, 91% of samples showed positive findings. *Cutibacterium* grew in 14% of NC samples. *Cutibacterium* grew in PC samples at an average of 4.0 ± 1.3 days, and all of these samples showed growth within 7 days. The time to positivity was significantly shorter ($P < .001$) and the strength of positivity was significantly higher ($P < .001$) in true-positive cultures compared with false-positive cultures.

Conclusions: This multi-institutional study suggests that different institutions may report highly consistent rates of culture positivity for revision shoulder arthroplasty samples with higher bacterial loads. In contrast, with lower bacterial loads, the results are somewhat less

This investigation was performed at 11 different institutions involved in the American Shoulder and Elbow Surgeons (ASES) Periprosthetic Joint Infection (PJI) Multicenter Group, with the Department of Orthopedics and Sports Medicine, University of Washington, Seattle, Washington, as the coordinating center.

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consistent. Clinicians should consider using a shorter time to positivity and a higher strength of positivity as adjuncts in determining whether a tissue culture sample is a true positive.

Level of evidence: Level IV; Case-Control Design; Diagnostic Study

Keywords: *Cutibacterium*; periprosthetic shoulder infection; periprosthetic joint infection; time to positivity; bacterial load; negative and positive control

Cutibacterium acnes is the most common bacterium isolated at the time of revision shoulder arthroplasty.[20,23] The spectrum of clinical presentations can vary widely, from gross infection with sinus tract formation and wound drainage to, more typically, insidious onset of pain and stiffness without obvious clinical signs of infection.[4,11] Owing to the fastidious nature of this bacterium, specialized media and prolonged culture holds are often required and recommended to isolate this organism.[3,15]

However, the interpretation of cultures positive for *Cutibacterium* can be difficult.[22] There exist several reports of positive culture findings from negative control (NC) specimens.[17,18] In addition, positive culture results have been obtained from primary arthroplasty cases without prior surgery.[9,13,22,25,27] Some surgeons will change surgical treatment and postsurgical antibiotic therapy if suspicion is high for obtaining positive *Cutibacterium* culture results,[26] whereas others will treat positive culture results as contaminants without changing treatment.[6,10] Furthermore, some institutions report other aspects of the cultures that may affect their interpretation, such as time to positivity and bacterial load.[1,7] Because there exists no standard of practice among surgeons and institutions regarding specimen handling, processing, culturing, and reporting of suspected shoulder periprosthetic infections during revision arthroplasty,[12] culture results from different surgeons and institutions may not be comparable.

Given the controversy surrounding the interpretation of positive *Cutibacterium* samples, we performed a multiinstitutional control study to compare laboratory differences in culture methodology and reporting. Our primary objective was to determine the rate of positivity of NC samples and positive control (PC) samples of varying bacterial loads of *Cutibacterium* at different institutions. Our secondary objective was to assess whether time to culture positivity and strength of culture positivity can assist in differentiating true positives from NC samples. We hypothesized that the rate of positivity with lower bacterial loads would vary significantly across institutions and that the time to positivity and the strength of positivity would vary significantly between PC and NC samples.

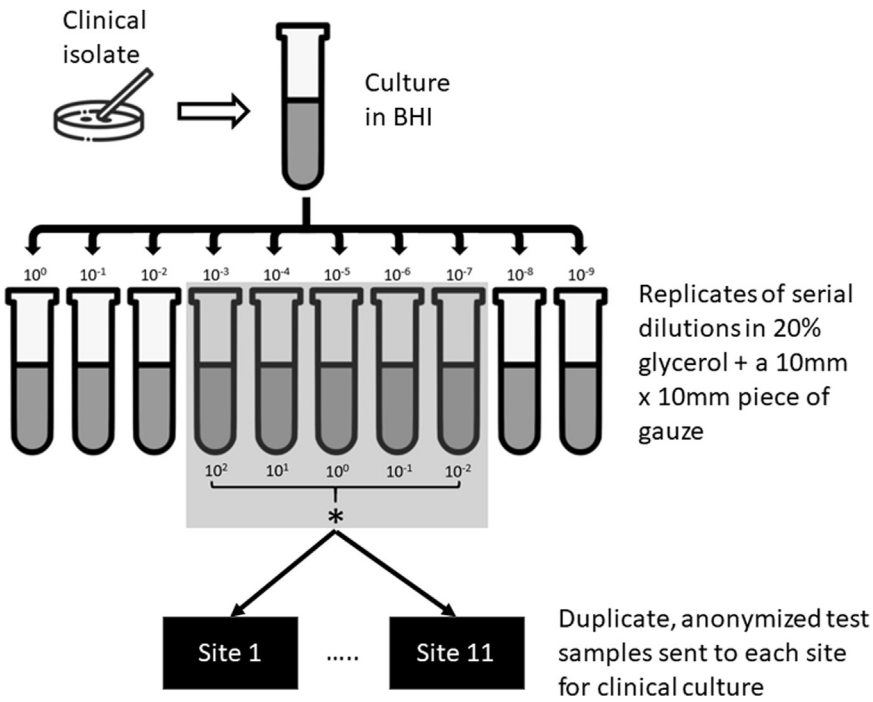

Figure 1 Growth of clinical isolate, serial dilutions, and distribution of samples. *BHI*, brain heart infusion. * 10^{0} references the amount of growth of the actual clinical specimen.

Materials and methods

Participating institutions

This study was a multi-institutional effort of the American Shoulder and Elbow Surgeons (ASES) Periprosthetic Joint Infection (PJI) Multicenter Group. Of the initial 22 institutions in the group, 11 institutions, across the United States, agreed to participate.

Study design and reporting

Each participating institution was provided with 12 blinded samples, including 10 PC samples (2 sets of 5 varying dilutions of the same *Cutibacterium* isolate) and 2 NC samples. Institutions were overnighted a set of samples and were instructed to test samples within a calendar week and to keep the specimens stored at −80°C until testing was performed. Samples were thawed at room temperature for 60 minutes prior to testing. Each institution was instructed to handle specimens and report results as if the specimens were from the operating room. Laboratories were asked not to alter any of their standard methods of culturing. Each institution was unaware of how many controls—or whether any controls—were included in the set of samples. The culturing methodology for each institution is included in Supplementary Table S1.

PC isolates

One strain of *C acnes* was isolated from the humeral canal tissue of a patient who underwent a total shoulder arthroplasty that was revised for pain and stiffness without any preoperative or intraoperative clinical or laboratory signs of infection. Of the 5 deep tissue samples obtained from this patient, all 5 were positive for *Cutibacterium*. By the International Consensus Meeting (ICM) definition of periprosthetic shoulder infection,[8] this patient was classified as having a "probable" prosthetic joint infection. The particular isolate was hemolytic with subtype IB by traditional subtyping[16] and H1 by single locus sequence typing.[24]

The isolate was grown in brain heart infusion broth under anaerobic conditions, and the bacterial density was quantified by the optical density at 600 nm (OD600) (Fig. 1). Ten serial dilutions were performed such that the lowest dilution contained approximately 0.1 colony-forming units per 100 μL of culture solution. One milliliter of 80% culture solution and 20% glycerol was combined with a 10-mm × 10-mm piece of gauze to replicate a tissue sample. This was placed in a 1.5-mL microcentrifuge tube and frozen at −80°C. Viability testing was performed, and a set of internal control specimens were cultured and sequenced to ensure that there was no contamination during the dilution process.

Five of the 10 dilutions were selected. As a clinically relevant reference, the bacterial load indicated by semiquantitative culture growth[1] of the middle of the 5 dilutions matched the semiquantitative culture growth of the actual clinical sample, referred to as the "10^0 dilution." Two dilutions above this (10^1 and 10^2) and two dilutions below this (10^{-1} and 10^{-2}) completed the set of 5 PC samples.

NC isolates

Similarly to the PC samples, the NC samples were produced by placing a 10-mm × 10-mm piece of gauze in a 1.5-mL microcentrifuge tube with 1 mL of sterile saline solution. This was frozen at −80°C.

Reporting of results

Each institution was asked to report culture positivity, time to culture positivity, and strength of culture positivity. Time to positivity was reported as the number of days by all 11 institutions. Strength of positivity was reported by 8 of 11 institutions using a semiquantitative method of growth in each of 4 quadrants of a culture plate. These results were categorized and reported as follows: 1-colony or broth growth only, 1-quadrant growth, 2-quadrant growth, 3-quadrant growth, or 4-quadrant growth. Numerical values were assigned to the culture results based on the number of quadrants with growth: Results with 1-colony or broth growth only were reported as a value of 0.1, whereas values of 1, 2, 3, and 4 were assigned to 1 quadrant, 2 quadrants, 3 quadrants, and 4 quadrants of growth, respectively.[15] In addition, each institution reported the media type and growth conditions used for the sample testing.

Statistical analysis

Time to positivity was treated as interval data, and strength of positivity was treated as ordinal data. Correlation testing was performed using Pearson correlation for time to positivity, whereas Spearman correlation was used for strength of positivity. The Kruskal-Wallis test was used to compare both time to positivity and strength of positivity between institutions. $P < .05$ was considered significant for all statistical testing.

Results

Rate of culture positivity

C acnes was isolated in all 22 samples (100% positivity) at the 4 highest PC dilutions (10^2, 10^1, 10^0, and 10^{-1}, with the 10^0 dilution matching the amount of growth of the actual clinical specimen) (Table I). The lowest dilution (10^{-2}) grew in 20 of 22 samples (91%). The NC samples grew *Cutibacterium* in 3 of 22 samples (14%) at 2 of the 11 institutions (18%).

Time to culture positivity

Shorter times to positivity were significantly related to bacterial load (Fig. 2). The mean time to positivity of all PC samples was 4.0 ± 1.3 days, and all of these samples showed growth within 7 days. Decreased bacterial concentration was associated with increased mean time to positivity (Spearmen $r = -0.9$, $P = .037$) (Table I). *Cutibacterium* growth in NC samples occurred at a mean of

Table I Rate of culture positivity, mean time to culture positivity, and mean strength of culture positivity of positive and negative control samples of *Cutibacterium*

Dilution	Rate of positivity, %	Time to positivity, mean ± SD, d	Strength of positivity, mean ± SD, quadrants
10^2	100	3.6 ± 1.2	2.4 ± 1.3
10^1	100	3.5 ± 1.0	1.9 ± 1.0
10^0	100	4.0 ± 0.9	1.4 ± 0.8
10^{-1}	100	4.5 ± 1.3	0.7 ± 0.6
10^{-2}	91	4.7 ± 1.6	0.3 ± 0.3
Negative control	14	8.3 ± 5.1	0.0 ± 0.3

SD, standard deviation.

8.3 ± 5.1 days, which was significantly longer than the mean time of the PC samples ($P < .001$).

There was significant variation in the mean time to positivity between the different institutions ($P = .038$) (Table II). When the 2 institutions that had positive growth in NC samples were compared with the other 9 institutions that had no positive growth in NC samples, the time to positivity of the PC samples was significantly shorter at those institutions without growth in NC samples than in those with growth (3.9 ± 1.2 days vs. 4.9 ± 1.1 days, $P = .001$). No identifiable differences in media type (all institutions performed anaerobic culture on blood agar) and growth conditions were noted between these institutions.

Strength of culture positivity

Bacterial load was significantly related to the number of quadrants showing growth (Fig. 2). An increased bacterial concentration was associated with increased strength of positivity (Spearman $r = 1$, $P < .001$). However, there was significant variation in the strength of positivity between the different institutions ($P < .001$). The strength of positivity was significantly lower in NCs compared with PCs ($P < .001$). No NC had >1 quadrant of *Cutibacterium* growth.

Discussion

Owing to the fastidious nature of *Cutibacterium* and potential for false-positive results, the interpretation and management of positive intraoperative culture findings, particularly in cases of revision shoulder arthroplasty, are controversial. This study used PC and NC samples of *Cutibacterium* to determine whether the interpretation of results across institutions is consistent and comparable. We also determined whether the time to culture positivity and the strength of culture positivity could aid in differentiation of PC and NC samples. Our results suggest that periprosthetic infections with higher *Cutibacterium* loads, even if indolent on clinical presentation, are likely to be reported similarly across different institutions. In contrast, periprosthetic infections with lower *Cutibacterium* loads may not produce similarly positive culture results across different institutions. Both the time to positivity and the strength of culture positivity may be helpful in interpreting the results of culture specimens obtained at revision arthroplasty.

Previous reports of positive culture findings obtained from NC samples have raised concerns that *Cutibacterium* grown from deep tissue samples may not be true-positive findings. Given that specialized incubation conditions and media, as well as longer culture hold times, are optimized to allow the growth of *C acnes*, previous studies incorporating NCs have reported substantial rates of positive culture results, presumably from the risk of contamination during the culturing and incubation process. Mook et al[17] reported a 13% false-positive rate for sterile gauze and Namdari et al[18,19] reported a false-positive rate of 15%-20% for sterile culture swabs exposed to air. Our study found a similar false-positive rate (14%) for the NC samples. There were no identifiable differences in the culturing protocols between those institutions that had false-positive growth and those that did not, suggesting that media type and growth conditions may not be as important as other potential explanations, such as handling of the samples during inoculation of the media. The ideal approach for identifying clinically significant culture results is as yet undefined.

Time to positivity has previously been suggested to have utility in differentiating so-called true- and false-positive culture results. Frangiamore et al[7] reported a significantly shorter time to positivity in a group of culture samples deemed to be "true positives" compared with a "probable contaminant" group (5 days vs. 9 days, $P = .002$). The results of our multi-institutional study showed a similar difference of 4 days between PC samples and NC samples. In addition, those institutions with positive growth in NC samples had a significantly longer time to positivity in PC samples compared with institutions without positive growth

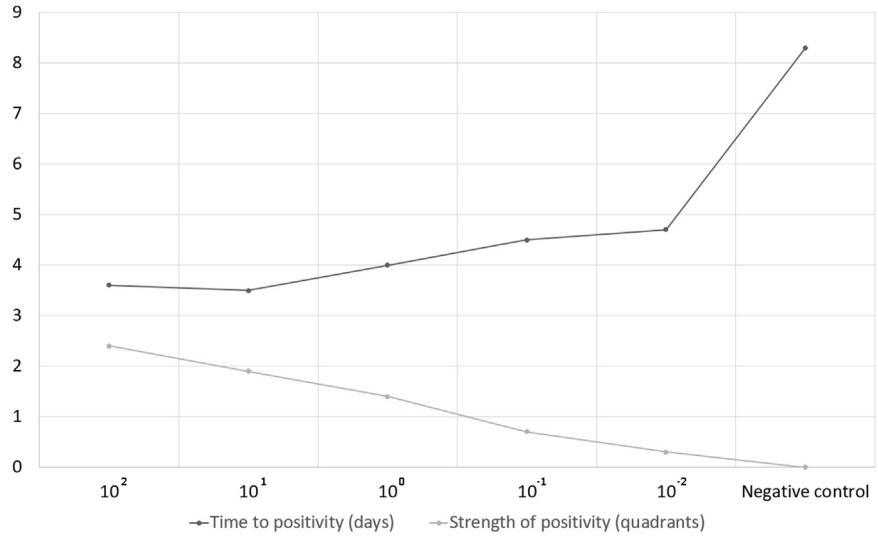

Figure 2 Time to positivity and strength of positivity relative to bacterial concentration.

in NC samples. The reason for this is not clear but may reflect differences in culturing between the institutions (whether media type or specimen handling) that cannot be elucidated with our methodology. We were unable to find any appreciable differences in terms of media types or growth conditions. Other factors such as specimen handling could account for positive growth in NC samples at these institutions.

The recommended duration of culture incubation is inconsistent across institutions; many institutions hold cultures for an extended period (>14 days),[3,15] whereas others suggest shorter incubation times.[5] The time-to-positivity data in this study suggest that culture results reporting shorter incubation times are more likely to be clinically relevant. However, differences in institutional protocols, equipment, and culturing could impact the rate of growth, and our results demonstrate that the time to positivity between institutions can vary (Table II). Some laboratories reported growth in all PCs by 3 days, whereas in others, up to 7 days was required. This limits our ability to make definitive conclusions on the duration of culture incubation.

The strength of culture positivity, as expected, correlated strongly with the bacterial load of the samples. The

Table II Comparison of time to positivity of positive control samples between institutions

	Time to positivity, mean ± SD, d	*P* value
Institution		.038
1	2.7 ± 0.8	
2	2.8 ± 0.6	
3	4.6 ± 0.7	
4	3.7 ± 0.9	
5	4.3 ± 1.7	
6	5.1 ± 0.3	
7	3.4 ± 0.5	
8	5.0 ± 1.1	
9	3.0 ± 0.0	
10*	4.8 ± 1.6	
11*	5.0 ± 0.0	
Institutions without growth in NC samples	3.9 ± 1.2	.001
Institutions with growth in NC samples	4.9 ± 1.1	

SD, standard deviation; *NC*, negative control.
The Kruskal-Wallis test was used for significance testing.
* Institution with positive growth in NC samples.

semiquantitative culture report may be useful in identifying clinically significant positive culture findings as no cultures of NCs grew past 1 quadrant. These results suggest that assessing the strength of positivity with semiquantitative culture reporting may be helpful in identifying clinically relevant culture positivity. This was suggested previously by MacNiven et al,[14] who reported a 4% positivity rate of NC air swabs but a 0% positivity rate for cultures with growth of > 1 colony on the culture plate

This study has limitations. First, not all laboratories had the capacity to perform sequence identification of positive samples, so it is unknown whether any of the PC samples could potentially have been a contaminant rather than true growth of the selected isolate. We did, however, sequence an internal set of control specimens to ensure that there was no contamination during the dilution process. Second, the threshold at which any particular dilution is considered clinically important (or not) is unknown, and further studies expanding laboratory findings to clinical outcomes are needed. Although the 10^0 dilution was used as a reference of the actual clinical sample, it is unknown whether the lower dilutions (10^{-1} and 10^{-2}) should be considered clinically relevant. Third, a hemolytic strain was selected for this study, which may not be representative of all strains found in periprosthetic shoulder infections. However, although the ratio of the incidence of hemolytic vs. non-hemolytic strains in shoulder PJI is not well known, there are multiple reports of shoulder PJI from hemolytic *C acnes*.[2,21] Use of other strains may have produced different results. Fourth, the sample size of this study (11 institutions) could be considered relatively small, but strong statistical significance of the results addressing the objectives of this study was demonstrated despite the limited sample size. Fifth, it is recognized that the diagnosis of a *Cutibacterium* periprosthetic infection in a post-revision shoulder requires the submission of multiple deep samples for culture because some samples from an infected shoulder may be culture negative. This study considered only the culture result for a single sample; in the clinical context, the combined culture results for all specimens may be important for guiding treatment. Sixth, we sent 2 NCs to each institution, which may not have been a sufficient sample size to make robust conclusions in interpreting the NC data. Finally, we are not able to determine the source of bacteria in NC samples that became culture positive.

Conclusion

The results of cultures of deep tissue and explant samples are a major element in the diagnosis of shoulder periprosthetic infection. This multi-institutional PC and NC study suggests that different institutions report a high degree of consistency for culture positivity for revision shoulder arthroplasty samples with higher bacterial loads, but lower bacterial loads may produce somewhat less consistent results. Both the time to culture positivity and the strength of culture positivity can be helpful adjuncts in identifying a clinically relevant positive culture result. Clinicians should consider using a shorter time to positivity and a higher strength of positivity as adjuncts in determining whether a culture that is positive for *Cutibacterium* is a true positive.

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Supplementary data

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