



Ten-day culture incubation time can accurately detect bacterial infection in periprosthetic infection in shoulder arthroplasty

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Background: *Cutibacterium acnes* is the most commonly isolated organism involved in periprosthetic shoulder infections. *C. acnes* has traditionally been difficult to isolate, and much debate exists over appropriate culture methods. Recently, our institution initiated a 10-day culture method using a Brucella blood agar medium to enhance anaerobic growth specifically for *C. acnes* in shoulder specimens.

Methods: A retrospective review of shoulder cultures from 2014–2017 of patients undergoing workup for possible infected shoulder arthroplasty was performed. Cultures were obtained in patients either preoperatively or intraoperatively at the time of revision. Presence of infection was determined based on at least 1 positive culture and treatment with either prolonged antibiotics, placement of an antibiotic spacer at the time of revision, or repeat surgical débridement.

Results: The records of 85 patients with 136 cultures were reviewed. Eighty-two patients had full records with at least 1-year clinical follow-up. Fifty-eight cultures were positive, with *C. acnes* as the most commonly recovered organism (57% of positive cultures). Clinical follow-up of patients with negative cultures found no incidence of missed periprosthetic infection.

Conclusions: Use of a 10-day culture incubation method to enhance anaerobic bacterial growth is able to accurately detect periprosthetic infection in the shoulder including those related to *C. acnes*. Our results suggest that by adopting more uniform culture methods, a shorter culture incubation time may be adequate. Ultimately, prospective studies with rigorous microbiologic methods are needed to best understand the clinical significance of unexpected positive bacterial cultures in shoulder arthroplasty.

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Once considered culture contaminant, *Cutibacterium acnes* (*C. acnes*, formerly *Propionibacterium acnes*) is now recognized as a common pathogen involving postoperative shoulder infections.^{12,13,20} This anaerobic, gram-positive, non-spore-forming bacillus has a propensity for sebum-rich, moist skin areas, such as the conjunctiva, face, scalp, and axilla. It is more commonly found in the shoulder region compared with other extremity sites, and infections due to this microbe disproportionately affect men.¹³ After shoulder arthroscopy, 19%–24% of patients were found to have incidental findings of deep cultures positive for *C. acnes*.^{12,20} Open

shoulder surgery positive culture rates were similarly around 20%.¹² Synovial fluid cultures obtained immediately after arthrotomy during primary shoulder arthroplasty were positive for *C. acnes* in 42% of patients.⁶ However, other studies with strict intraoperative specimen collection criteria found positive cultures in only 3.125% of patients.⁹ Recently, the International Consensus Meeting on Musculoskeletal Infection stated that the relevance of unexpected positive cultures in the setting of shoulder arthroplasty is unknown.⁵

Several studies^{1,8,19} have suggested extended incubation to maximize *C. acnes* recovery from surgical specimens and joint fluids. Increasing incubation time may lead to increased contamination in culture specimens. Butler-Wu et al¹ found that incubation beyond 13 days was associated with increased recovery of non-diagnostic *C. acnes* isolates, whereas “clinically significant” *C. acnes*–infected events were observed when the organism was

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recovered in cultures incubated 7.3 ± 2.6 days. Similarly, Frangi-more et al⁴ noted that in cases of probable infection cultures, the organism was detected within an average of 5 days of incubation compared with 9 days for probable contaminants. As with any laboratory testing, the attempts to increase the sensitivity of *C acnes* detection, such as extending the incubation time for both aerobic and anaerobic cultures,¹ use of enriched broth,¹⁹ or using improved sampling procedures,²² may come at the expense of clinical specificity as well as increasing laboratory labor and cost. Despite evidence suggesting that longer incubation may be associated with increased contamination, this risk may be overshadowed by the purported fear of undiagnosed infection if shorter incubation times are employed. There is currently no consensus regarding the type of incubation that best detects true infection caused by *C acnes*.⁵ Additionally, use of advanced sampling techniques may come at the risk of further increased false-positive rate.⁵

Thus, the purpose of this study was to evaluate the impact of an enhanced culture protocol—whereby anaerobic culture medium was incubated for 10 days—on the recovery of *C acnes* in shoulder cultures collected from patients with clinical suspicion of infection following shoulder arthroplasty. Our institution implemented this culture method for all shoulder specimens in 2014 with the goal of enhanced recovery of anaerobic bacteria such as *C acnes* in shoulder specimens. We hypothesized that this protocol would effectively detect clinically relevant infections without a significant increase in missed infections.

Materials and methods

In this retrospective study, the medical records of patients who had a shoulder specimen culture between 2014–2017 at our institution were reviewed. A review of the microbiology laboratory census during this time period identified 175 patients who had at least 1 culture obtained from the shoulder joint. Those without a history of shoulder arthroplasty were excluded. All those with a history of shoulder arthroplasty needed at least 1 year of clinical follow-up after culture sampling. We initially identified 89 patients with cultures obtained in the setting of a shoulder arthroplasty. Based on these inclusion and exclusion criteria, a total of 136 culture events in 85 patients were identified and comprised our cohort. All cultures were obtained in preparation for revision arthroplasty (either via a joint aspiration or arthroscopic tissue biopsy) or at the time of revision arthroplasty based on the surgeons' clinical suspicion of infection and/or intraoperative findings. The typical practice of the shoulder surgeons at our institution was only to obtain cultures when the clinical suspicion for infection was significant, either preoperatively or intraoperatively. Cultures were not routinely obtained for all revision cases during the time in which these samples were collected. During this time period, 224 revision shoulder arthroplasties were performed at our institution.

Preoperative suspicion for infection was determined based on the presence of at least 2 of the following criteria: (1) Clinical concern as documented in the medical record,¹¹ (2) skin erythema, (3) presence of a draining sinus, (4) elevated inflammatory markers (erythrocyte sedimentation rate, C-reactive protein),¹⁵ and (5) humeral osteolysis or loosening on radiographs.¹⁶ Intraoperative suspicion of infection was at the treating surgeon's discretion. Clinical charts and radiographs were reviewed by a shoulder and elbow fellow and fellowship-trained surgeon to determine the eventual clinical course of the patient.

The diagnosis of a periprosthetic shoulder infection was made based on review of the clinical charts as well as culture results. Patients were defined as infected cases with at least 1 positive culture and any of the following: (1) treatment with explantation and/or antibiotic spacer at time of first revision, (2) treatment with

antibiotics for an extended course (>4 weeks), or (3) subsequent surgical débridement/prosthesis removal after culture results. Similarly, patients with positive cultures who did not undergo the above treatments were categorized as treated as contaminant. Shields et al²¹ note that because of its indolent nature, the diagnosis of *C acnes* infection can be difficult and must combine preoperative clinical and radiographic data and laboratory and culture data. At the time of the culture collection, there were no agreed on standards for diagnosis of periprosthetic shoulder infection and our institution was not uniform in the diagnostic workup and management of periprosthetic infection.

Laboratory methods

All specimens were processed by our institution's microbiology laboratory in a class-2 laminar flow biological safety cabinet. Specimen Gram stains were prepared on all sterile fluids (joint fluid and synovial fluid), sterile tissue, and explanted hardware. After early 2014, a modification was made to the standard laboratory protocol at our institution. In addition 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. Any organism recovered in culture was identified according to the laboratory standard operating procedures, involving a combination of Gram staining, phenotypic profiling, biochemical analysis (catalase, indole), and MALDI-TOF MS (Bruker Biotyper, Billerica, MA, USA) testing.

Results

Overall, 136 separate culture events occurred in 85 patients who met inclusion/exclusion criteria. Basic demographics are listed in [Table I](#). Of these 85 patients, 52 (61.2%) presented with criteria concerning for infection ([Table II](#)). The other 33 patients had cultures taken intraoperatively because of intraoperative concern of infection. Of the 136 cultures obtained, 101 (74.3%) were tissue cultures, and the remaining were joint aspiration cultures. Intraoperative cultures taken during the time of revision surgery were obtained in 57 of the 136 culture events (41.9%). The number of cultures obtained varied from 1 to 8, with a median of 3 cultures obtained per event.

At least 1 culture turned positive in 58 of 136 events (42.6%), with *C acnes* being the most frequently isolated organism ([Table III](#)). [Table IV](#) shows the results of number of samples obtained at either preoperative workup or revision with number of positive cultures. When analyzing the initial culture event (either from preoperative workup or initial revision surgery) for each given patient, at least 1 culture turned positive in 34 of 85 patients (40%), again with *C acnes*

Table I
Demographics (n = 85)

Age, yr, mean \pm SD	63.1 \pm 11.1
Sex, male	44 (51.8)
Hemiarthroplasty	6 (7.1)
Anatomic total shoulder	48 (56.5)
Reverse shoulder	27 (31.8)
Antibiotic spacer	4 (4.7)

SD, standard deviation.

Unless otherwise noted, values are n (%).

Table II
Frequency of patients with infection concerns preoperatively

Infection concern	n (%)
Erythema	10 (11.8)
Draining sinus	7 (8.2)
Elevated ESR	15/38 (39.5)
Elevated CRP	11/38 (28.9)
Glenoid osteolysis	22/61 (36.1)
Humeral osteolysis	37/65 (56.9)

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

being the most frequently isolated organism. Only 4 of 35 aspiration cultures (11%) turned positive, compared to 54 of 101 tissue cultures (53%).

Culture events with at least 1 positive culture were treated as an infection (either with prolonged antibiotics or subsequent surgical débridement/explant) in 40 of 58 events (69.0%). The decision to treat with antibiotics was at the discretion of the treating surgeon in conjunction with consultation from our infectious disease service. Unfortunately, at the time of this study, there was no agreed on standard for treatment of positive cultures. Of the 18 events that were not treated as infection, 15 were single isolated cultures of either *C acnes* or *Staphylococcus epidermidis*. None of the patients whose positive cultures were considered to be a contaminant presented back to our institution with signs of periprosthetic infection or required revision surgery. Interestingly, of the 33 patients in whom there was only intraoperative concern without any other preoperative suspicion for infection, only 1 patient had a true infection that required subsequent débridement. For this subset of patients, none of them underwent an explant with spacer at the time of first revision. *C acnes* accounted for 57% of the positive cultures isolated in this study, consistent with previous literature. *C acnes* isolates were exclusively grown on anaerobic media, including BBA.

There were 4 culture samples obtained in patients with previously known periprosthetic infection being treated at the time of culture sampling with antibiotics that did not yield microbial growth. No patient with a negative culture presented with subsequent development of periprosthetic infection (Table V). Similarly, for the first culture sampled in the cohort of patients, 24 of 34 (71%) were treated as an infection. Importantly, none of the patients who had negative cultures at the time of first culture sampling went on to develop subsequent periprosthetic infection.

Discussion

Periprosthetic shoulder infection, although rare, remains a devastating and diagnostically challenging complication. The most common pathogen identified is *C acnes*.¹⁸ *C acnes* is a slow-growing, gram-positive anaerobe located subdermally in hair follicles that is commonly identified within normal subdermal tissue at the time of shoulder arthroplasty.^{1,6,9,12,13,20,21} Growing *C acnes* on culture medium is challenging because of its anaerobic nature, and most experts recommend an “extended incubation” period to help

Table III
Frequency of bacterial micro-organisms in positive cultures

Bacterial micro-organism	n (%)
<i>Cutibacterium acnes</i>	33 (57)
<i>Staphylococcus epidermidis</i>	8 (13.8)
<i>Staphylococcus aureus</i>	5 (8.6)
Other*	12 (20.6)

* *Micrococcus*, *Streptococcus mitis*, *Staphylococcus intermedius*.

Table IV
Number of samples obtained with number of positive cultures

Number of samples obtained	Number of positive cultures							Total	
	0	1	2	3	4	5	6		7
1	37	6							43
2	9	2							11
3	8	2	2	3					15
4	8	5	0	1	1				15
5	8	7	2	2	3	1			23
6	4	2	4	1	0	0	0		11
7	1	2	5	1	1	2	1	0	13
8	2	1	0	0	0	0	0	0	3
9	0	0	0	0	0	0	1	1	2
Total	77	27	13	8	5	3	2	1	136

promote growth. To date, there is a lack of consensus on the optimal culture method or incubation time; several methods exist, and current recommendations suggest holding cultures for at least 14 days. Unfortunately, extending incubation may also lead to increased contamination, decreasing the ability for a culture to predict the presence of infection. Recently, our institution's microbiology laboratory instituted a 10-day culture method using a solid culture medium, a BBA culture plate, to enhance anaerobic growth. The findings of our retrospective study in a cohort of patients with suspicion for periprosthetic infection of the shoulder found this method to be accurate in aiding diagnosis of periprosthetic infections. Importantly, no patients with a negative culture developed a subsequent periprosthetic infection.

The significance of *C acnes*–positive cultures has been debated. It is unclear whether *C acnes* is part of a normal shoulder microbiome or whether it is introduced at the time of surgery. It is becoming more clear that *C acnes* is not always a culture contaminant but can be the micro-organism responsible for periprosthetic infection in many instances. Many authors have theorized that *C acnes* is not native to the shoulder, but rather introduced at the time of surgery.^{3,10,14} Falconer et al³ obtained cultures from multiple sites during primary shoulder arthroplasty and determined the rate of positive cultures at each site. They concluded that *C acnes* was introduced into the shoulder during surgery because of the preponderance of positive cultures obtained from superficial and subdermal locations, compared with the relative paucity of positive cultures collected from deep tissues.³ The same instruments were used for specimen collection, raising the potential for contamination. In this and similar studies, different culture methods and incubation times have been used, making it more difficult to clearly determine which positive cultures are significant and which are contaminants. It is interesting that no infections were reported in the Falconer study, despite the high number of positive cultures.

Qiu et al¹⁷ recently investigated the presence of a shoulder microbiome by analyzing tissue samples using 16S ribosomal RNA (rRNA) gene sequencing and polymerase chain reaction amplification to identify the presence of bacteria. They used strict tissue specimen collection techniques, excluded patients with prior shoulder surgery or concern for infection, and used control samples to exclude likely contaminants. Of the 136 samples collected, only 53 (39%) showed RNA of bacterial origin. The most commonly

Table V
Distribution of treatment regimens based on culture results

Culture	Treated as infected	Treated as not infected
Positive	40	18
Negative	4	75

identified bacteria were 2 *Acinetobacter* species and 1 *Oxalobacteriaceae* species. *Cutibacterium* was identified in only 1 skin sample on 1 male patient. They concluded that the shoulder likely has a microbiome, and *Cutibacterium* is likely not part of this microbiome. It is unclear what the function of this microbiome may be or whether the RNA was from live or dead bacteria. This does lend some support to the idea that *C. acnes* is introduced into the deep tissues at the time of surgery. It is interesting to note, however, that only 39% of the samples were positive for any bacteria, and it is unclear whether these positive samples were from a handful of patients or evenly distributed across all shoulders sampled. Additionally, the cell wall of *C. acnes* makes intracellular material difficult to extract, making polymerase chain reaction amplification of rRNA less useful in detecting *C. acnes*.⁷ It is difficult to conclude whether a true shoulder microbiome exists with the available literature at this point.

Extending incubation for anaerobic growth can lead to an increased rate of culture contaminants less likely to be clinically relevant.^{1,4} This can be due to increased handling of cultures and improper culture media that may desiccate during the extended time. Some methods of enhancing incubation include use of anaerobic broth, specific media that can survive beyond 5 days, use of blood culture bottles, and use of different media for enhancing anaerobic growth. Mook et al.¹² found positive cultures in 13% of gauze specimens obtained in the operating room at the time of surgery as well as 18% of primary shoulders at the time of surgery. They also noted that cultures continued to grow with time and suggested that contamination was more likely when cultures were held longer than 14 days.

Other studies describe similar findings but are not specific in terms of the type of extended incubation used. Extended incubation can lead to increased costs and decrease accuracy of cultures, complicating clinical decision making when considering whether to treat or how to appropriately treat. Diagnosis of periprosthetic shoulder infection is complex and includes consideration of clinical, radiologic, and bacteriologic elements. Prior studies report that the majority of, if not all, cultures turn positive by or around day 10, with the average time to positive culture of 8–9 days.^{2,3,10,23} Matsen et al.¹⁰ also noted that anaerobic media cultures had a shorter time to positivity, and Butler-Wu et al.¹ noted that culture positivity on sheep blood agar was highly correlated with periprosthetic joint infection. Our study found that using a BBA plate with a 10-day incubation period allowed to accurately detect periprosthetic infection without any undiagnosed infections.

In our experience, the addition of a BBA plate to the existing laboratory procedure for shoulder cultures is inexpensive (approximately US\$5 per plate) and results in a minimal increase in the overall labor time, compared with using sophisticated sampling procedures or broth enrichment techniques described in previous studies.^{19,22} Although previous studies proved that extended incubation (>7 days) is necessary to recover *C. acnes*, there was no agreement on the optimized culture time and condition.^{1,8,19} In the current study, a 10-day incubation time was applied to anaerobic media as described. The present protocol also required the anaerobic media to be placed in GasPak bags after 3 full days if there was no growth, and not examined until day 10. The design was meant to balance the sensitivity and specificity for obtaining clinically significant microbes.

Our study has several limitations. First, it is a retrospective study, and determining the preoperative level of suspicion depended on available data in the medical chart. Similarly, clinic follow-up was dependent on patients returning for follow-up, but patients were excluded if they did not have clinical follow-up for at least 1 year after their culture was obtained. Defining true infection was also difficult as there is still debate on the definition

of shoulder periprosthetic infection. At the most recent International Consensus Meeting on Orthopaedic Infections, the only definitive signs of true infection were a draining sinus or unexpected wound drainage, and gross purulence encountered at the time or revision surgery.⁵ This is a particularly rare finding in shoulder infections, especially those caused by *C. acnes*. The typical *C. acnes* infection has a very indolent course and rarely presents with overt signs of infection. Other signs, such as radiographic loosening, elevated inflammatory markers, pain, stiffness, skin erythema, and fever are less specific and useful for determining infection. Given this debate, we felt that our criteria of a positive culture along with treatment with débridement, explant and spacer placement, and/or extended antibiotic treatment were appropriate. Additionally, the Consensus Meeting was conducted after the study period. Treatment decision making was also at the discretion of the treating surgeon without uniformity in criteria for treating as infection or contaminant.

Conclusion

The use of a 10-day culture incubation time using BBA incubated anaerobically is an effective diagnostic tool for periprosthetic shoulder infection, especially with *C. acnes*. Importantly, using this culture incubation time, we did not identify any cases where clinically relevant infections were overlooked. Our results suggest that by adopting more uniform culture methods, a shorter culture incubation time may be adequate. Ultimately, prospective studies with rigorous microbiologic methods are needed to best understand the clinical significance of unexpected positive bacterial cultures in shoulder arthroplasty.

Disclaimer

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