A commentary by Grant Garrigues, MD, is linked to the online version of this article at jbjs.org.

Characterizing the Propionibacterium Load in Revision Shoulder Arthroplasty

A Study of 137 Culture-Positive Cases

Zahab S. Ahsan, MD, Jeremy S. Somerson, MD, and Frederick A. Matsen III, MD

Investigation performed at the Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, Washington

Background: Propionibacterium is commonly recovered from explants or surrounding tissues in revision shoulder arthroplasty. Rather than attempting to differentiate a true infection from a false-positive result on the basis of the number of positive cultures, we characterized the amount of these bacteria in each specimen and shoulder.

Methods: The study included 137 revision shoulder arthroplasties from which a minimum of 4 specimens had been submitted for culture and at least 1 was positive for Propionibacterium. Standard microbiology procedures were used to assign a semiquantitative value (0.1, 1, 2, 3, or 4), called the *Specimen Propi Value,* to the amount of growth in each specimen. The sum of the Specimen Propi Values for each shoulder was defined as the Shoulder Propi Score, which was then divided by the total number of specimens to calculate the Average Shoulder Propi Score.

Results: The number and percentage of positive specimen-specific cultures (of material obtained from the stem explant, head explant, glenoid explant, humeral membrane, collar membrane, other soft tissue, fluid, or other) per shoulder ranged from 1 to 6 and 14% to 100%. A high percentage of specimens (mean, 43%; median, 50%) from the culture-positive shoulders showed no growth. Only 32.6% of the fluid cultures were positive in comparison with 66.5% of the soft-tissue cultures and 55.6% of the cultures of explant specimens. The average Specimen Propi Value (and standard deviation) for fluid specimens (0.35 ± 0.89) was significantly lower than those for the soft-tissue (0.92 ± 1.50) and explant (0.66 ± 0.90) specimens ($p < 0.001$). The Shoulder Propi Score was significantly higher in men (3.56 ± 3.74) than in women (1.22 ± 3.11) ($p < 0.001$). Similarly, men had a significantly higher Average Shoulder Propi Score (0.53 ± 0.51) than women (0.19 ± 0.43) ($p < 0.001$).

Conclusions: This investigation suggests that Propionibacterium is unevenly distributed within culture-positive revised shoulders. As a result, the specimen number and source (explant, soft tissue, or fluid) have major influences on the culture results for a revised shoulder arthroplasty. We found no evidence that suggested useful threshold values for defining a true infection.

Level of Evidence: Diagnostic Level III. See Instructions for Authors for a complete description of levels of evidence.

Peer Review: This article was reviewed by the Editor-in-Chief and one Deputy Editor, and it underwent blinded review by two or more outside experts. The Deputy Editor reviewed each revision of the article, and it underwent a final review by the Editor-in-Chief prior to publication. Final corrections and clarifications occurred during one or more exchanges between the author(s) and copyeditors.

Propionibacterium is commonly grown on culture of specimens harvested during revision shoulder arthroplasties performed for pain, stiffness, or component loosening, even though a patient may not show obvious clinical, laboratory, or radiographic signs of periprosthetic infection[1-5]. Some authors have tried to develop criteria for distinguishing "true infections," "false-positive cultures," and "contamination" in such cases on the basis of clinical signs and number of positive

Disclosure: There was no external funding for this study. On the **Disclosure of Potential Conflicts of Interest** forms, *which are provided with the online version of the article,* one or more of the authors checked "yes" to indicate that the author had a relevant financial relationship in the biomedical arena outside the submitted work.

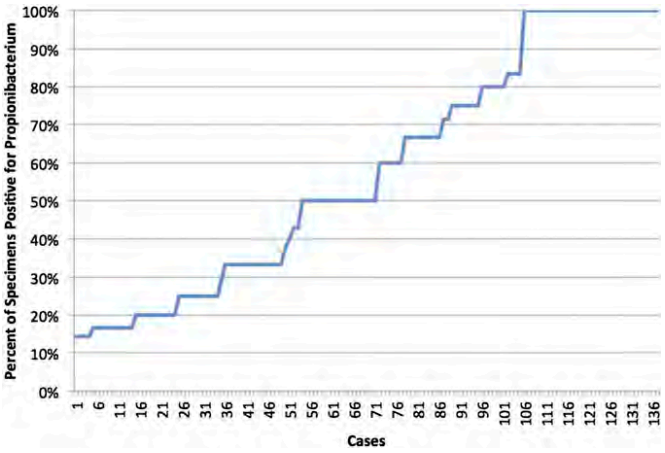

Fig. 1

The continuous distribution of the percentage of specimens that were culture-positive among 137 culture-positive revised shoulders. Note the absence of a cutoff value that would suggest a threshold for defining a true-positive result.

cultures[6]. However, because the clinical signs lack sensitivity and specificity for predicting Propionibacterium infection and because often as few as 2 specimens are submitted for culture, these criteria have proven difficult to apply consistently[7-12].

Our experience indicates that there is no obvious, practical threshold for culture results obtained at the time of revision shoulder arthroplasty that can differentiate a true Propionibacterium infection from a false-positive result. Instead, the results vary from a minimal presence of Propionibacterium (a small amount of growth from one of several specimens submitted) to an overwhelming presence (heavy growth in a high percentage of the specimens). Thus, rather than attempting to refine a definition of periprosthetic Propionibacterium infection for shoulder arthroplasty akin to what has been done for hip and knee arthroplasty (based, for example, on the presence or absence of pathogens in at least 2 separate tissue or fluid samples)[13], we suggest that studies of revision shoulder arthroplasty would be better informed if they (1) routinely included culture data from at least 4 tissue or explant specimens and (2) used measures of the bacterial load in the specimens.

In this investigation of patients who had had at least 1 positive culture of specimens obtained during a revision shoulder arthroplasty, we tested the hypotheses that (1) the degrees of culture positivity vary widely among specimens harvested during same shoulder revision, (2) the degrees of positivity are higher for cultures of humeral and glenoid component explant and soft-tissue specimens than for fluid cultures, (3) the total of the degrees of positivity for all specimens from a shoulder are associated with the patient's sex, and (4) there is no obvious threshold for culture results that allow a distinction between a true-positive and a false-positive finding of infection.

Materials and Methods

When we revise a failed arthroplasty because of pain, stiffness, or component loosening (but without obvious clinical evidence of infection), we routinely harvest specimens of explants, soft tissues, and/or joint fluid with individual sterile instruments before intravenous antibiotic administration. These specimens are submitted for culture according to a defined protocol to optimize the opportunity of recovering Propionibacterium[2,14]. Specifically, all specimens are cultured for 3 weeks in blood agar (Trypticase soy agar with 5% sheep blood), chocolate agar, Brucella agar (with blood, hemin, and vitamin K), and brain-heart infusion broth. All media, with the exception of the Brucella agar, are incubated at 37°C with 5% CO_2 for 28 days. Brucella agar plates are incubated anaerobically at 37°C for 28 days. Media are visually examined daily for growth, and the culture plates are opened only if growth is noted.

This study was approved by our institutional review board (#51461). In a review of the 223 revision arthroplasties performed by the senior author between April 10, 2007, and February 9, 2016, 168 shoulders (75%) were found to have at least 1 culture that was positive for Propionibacterium. For 31 shoulders, 3 or fewer specimens were submitted for culture, and these shoulders were omitted from this analysis, leaving 137 culture-positive shoulders, in 34 women (average age [and standard deviation], 58.2 ± 13.2 years; range, 27 to 78 years) and 103 men (average age, 61.4 ± 10.8 years; range, 22 to 81 years; p = 0.27), from which at least 4 specimens had been submitted for culture[2].

As is the case at many centers, our microbiology laboratory reports positive cultures in semiquantitative terms (growth in broth only, 1 colony, 1+, 2+, 3+, or 4+) on the basis of the findings at the end of the 3-week culture period. "Broth only" indicates that only the broth was culture positive (the streaked plate did not show growth), and "1 colony" indicates that only 1 bacterial colony was noted on the streaked plate. The scale of 1+ to 4+ indicates the number of quadrants (1 to 4) that showed growth on a plate streaked with the standard technique[15]. As reported previously, we assigned numerical values (Specimen Propi Values) to the semiquantitative Propionibacterium culture results: 0.1 (broth only), 0.1 (1 colony), 1, 2, 3, and 4 (1+, 2+, 3+, or 4+, respectively)[2].

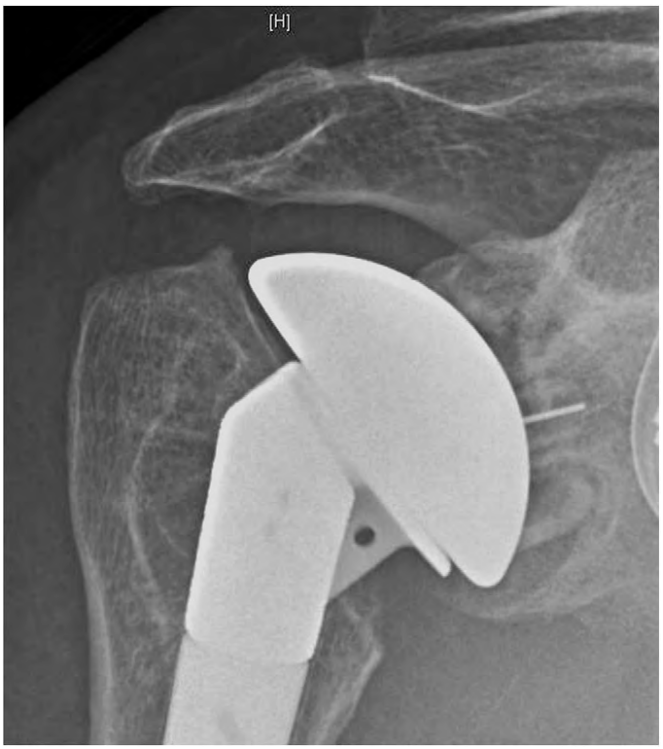

Fig. 2

The presenting anteroposterior radiograph of a 60-year-old man with no clinical signs of infection. The radiograph suggests the possibility of aseptic loosening.

TABLE I Culture Results from a Patient with Three Successive Revision Shoulder Arthroplasties*

	Explant Specimens			Soft-Tissue Specimens							
	Humeral Stem	Humeral Head	Glenoid	Humeral Membrane	Collar Membrane	Other	Fluid Specimen	Other Specimen	Total No. of Specimens	Shoulder Propi Score	Average Shoulder Propi Score
1st revision											
No. of specimens	1	1	1			3	1	1	8		
Specimen Propi Value	0.1	1	1			1.1	0	0.1		3.3	0.41
2nd revision											
No. of specimens	1	1		3	1		1		7		
Specimen Propi Value	1	0		1.2	0		0			2.2	0.31
3rd revision											
No. specimens	1	1		2	1	3			8		
Specimen Propi Value	2	1		2	0	0				5.0	0.63

*The fluid cultures were negative despite the presence of Propionibacterium in multiple other specimens obtained at the same procedure. Also, Propionibacterium was not evenly distributed in the shoulder; some explant and soft-tissue cultures were positive while others obtained at the same time were negative. Finally, the Shoulder Propi Score and the Average Shoulder Propi Score did not lessen with repeated surgical revisions.

The Specimen Propi Values were summed for each type of specimen (humeral stem explant, humeral head explant, glenoid explant, collar membrane [between the modular head and stem], humeral membrane [between the humeral stem and humeral bone], other soft tissue, fluid, or "other") from each shoulder. Each tissue specimen was harvested with a previously unused sterile rongeur, with care taken to avoid contact with other tissue or implants. The explant specimens were obtained by vortexing the retrieved implant in 3 mL of sterile saline solution. The fluid specimens were obtained with a sterile syringe. The Specimen Propi Values for all of the specimens from a particular shoulder were summed to derive the Shoulder Propi Score for that shoulder[2]. If ≥2 different colony types or species were reported for the same specimen, the assigned values were added. We have noted that prior clinical studies commonly refer to all species of Propionibacterium as "*P. acnes,*" even though other species of Propionibacterium are found in specimens retrieved during shoulder revisions[16]. In this analysis, laboratory reports of *P. acnes, P. avidum, P. granulosum, P. humerusii, P. species,* and "presumed" Propionibacterium were all counted as Propionibacterium. Because of the small numbers of organisms other than *P. acnes,* we did not separately analyze the data for the different species. Because the Shoulder Propi Score was influenced by the number of culture specimens submitted, we used a third metric, the Average Shoulder Propi Score, defined as the Shoulder Propi Score divided by the total number of specimens from that shoulder submitted for culture.

The average Specimen Propi Values (and standard deviation) for the different types of specimens (e.g., explant, soft tissue, and fluid) were determined, and the significance of the differences between the results of fluid cultures and (1) explant-specimen cultures and (2) soft-tissue cultures were analyzed with an unpaired t test. The Shoulder Propi Score and Average Shoulder Propi Score were determined for male and female patients, and the significance of the differences was analyzed with an unpaired t test. Significance was defined as $p < 0.05$. Because of the possibility that the load of Propionibacterium was related to age-dependent hormonal changes,

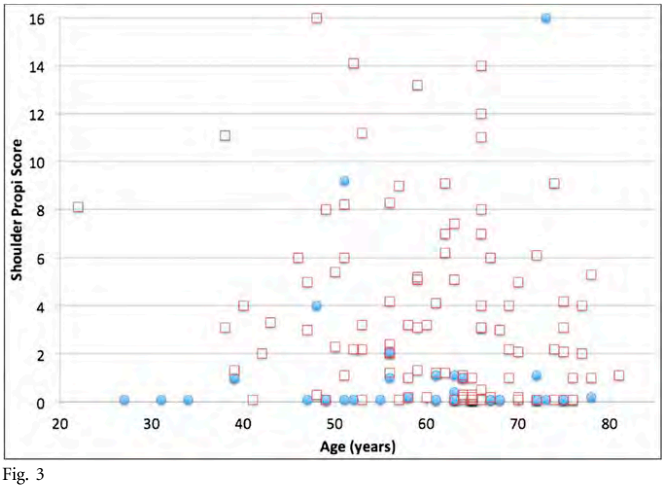

Fig. 3

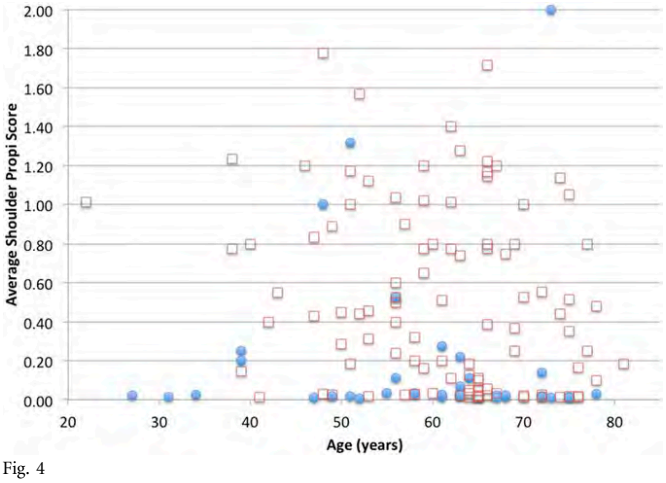

Fig. 4

Fig. 3 The distribution of Shoulder Propi Scores by patient sex (males represented by squares and females, by circles) and age. The score shows a dramatic relationship with sex but none with age. Note also the absence of a cutoff value that would suggest a threshold for defining a true-positive result.

Fig. 4 Distribution of Average Shoulder Propi Scores by patient sex (males represented by squares and females, by circles) and age. The score shows a dramatic relationship with sex but none with age. Note also the absence of a cutoff value that would suggest a threshold for defining a true-positive result.

we also compared the distribution of Shoulder Propi Scores by patient age and sex.

Results

An average of 6.62 ± 2.61 specimens from female patients and 6.69 ± 2.06 specimens from male patients were cultured ($p = 0.88$). On average, 3.1 of the specimens were of soft tissue, 2.5 were from explants, and 1.1 were of joint fluid. The number and percentage of positive specimen-specific cultures per shoulder ranged from 1 to 6 and 14% to 100% (Fig. 1). Neither of these parameters demonstrated a threshold that might be used to identify true-positive culture results. Propionibacterium was found to varying degrees in specimens of different types and from different locations. Shoulders with positive cultures had a high percentage of specimens that showed no growth (mean, 43%; median, 50%). Figure 2 and Table I show an example of uneven Propionibacterium distribution. In this case, the Propionibacterium was refractory to multiple single-stage revisions; the results of the preoperative laboratory studies of serum inflammatory markers were all normal.

Only 32.6% of the fluid cultures were positive in comparison with 66.5% of the soft-tissue cultures and 55.6% of the cultures of the explant specimens. The average Specimen Propi Value for fluid (0.35 ± 0.89) was significantly lower than that for soft tissue (0.92 ± 1.50) and explant specimens (0.66 ± 0.90) ($p < 0.001$). Men had a significantly higher mean Shoulder Propi Score (3.56 ± 3.74) than women (1.22 ± 3.11) ($p < 0.001$), but patient age did not have a significant effect on the score (Fig. 3). Similarly, men had a significantly higher Average Shoulder Propi Score (0.53 ± 0.51) than women (0.19 ± 0.43) ($p < 0.001$), but patient age did not have a significant effect (Fig. 4). The Shoulder Propi Score and Average Shoulder Propi Score varied among the shoulders that were culture-positive for Propionibacterium, without a clear threshold above which a true-positive result could be defined with confidence.

Discussion

The Workgroup of the Musculoskeletal Infection Society has developed criteria for periprosthetic infections of the hip and knee[13]. Investigators have had difficulty applying similar criteria to periprosthetic infections of the shoulder, largely because most shoulder infections are of the "stealth" type, presenting as pain, stiffness, and apparent aseptic loosening without the features characteristic of obvious infection. This difference is likely due to the fact that the most common bacteria recovered from the sites of failed shoulder arthroplasties are low-virulence organisms, such as Propionibacterium[4]. The presence of Propionibacterium may be overlooked because the presentation is subtle, special culture techniques are required for reliable detection[2], surgeons often collect a small number of specimens for culture[2], and the infections often present years after the index arthroplasty[3]. For these reasons, the development of a practical definition of periprosthetic shoulder infection has been difficult. Thus, the evaluation and management of failed shoulder arthroplasties have been complicated by concerns about "unexpected positive cultures," "false-positive cultures," and "contamination."[5,6,10,11,17-21]

Up to this point, efforts to interpret the results of cultures of specimens obtained at the time of revision surgery have been based on the number of cultures that were positive (for example, it has been suggested that ≥2 cultures positive for Propionibacterium is clinically relevant)[7-12]. The problem with this approach is that the number of specimens that are culture-positive depends on (1) the nature of the specimens (explant, tissue, or fluid), (2) how many specimens are submitted for culture, (3) what media are used for culture, and (4) how long the cultures are observed[2]. Inconsistency among these variables confuses attempts to compare data among patients and among studies and confounds efforts to establish guidelines for diagnosis and management of shoulders with possible periprosthetic infection.

This study presents three straightforward metrics that can characterize the results of cultures, assuming that multiple specimens are harvested and that the specimens are cultured for Propionibacterium on aerobic and anaerobic media. The Specimen Propi Value is assumed to be a reflection of the bacteria burden in each specimen submitted for culture, with an indication of whether the specimens were obtained from explants, soft tissue, or joint fluid. The Shoulder Propi Score indicates the total of the Specimen Propi Values for all specimens obtained from a specific shoulder. The Average Shoulder Propi Score indicates the total of the Specimen Propi Values for specimens obtained from a specific shoulder (the Shoulder Propi Score) divided by the number of specimens submitted for that shoulder. While not proven in this study, shoulders with a high Average Shoulder Propi Score seem likely to have larger numbers and concentrations of bacteria (i.e., a higher bacterial load) than those with a lower score.

It is important to note that, within the same shoulder, the Specimen Propi Values may be 0 for some specimens and highly positive for others collected during the same surgery. Soft-tissue and explant specimens had higher rates of culture positivity than joint fluid; this observation is consistent with the tendency of Propionibacterium to reside in a biofilm rather than existing in planktonic form in joint fluid that is accessible by aspiration. Conclusions about the presence of Propionibacterium in a shoulder need to be based on the number and nature of the specimens submitted for culture.

While it has been previously noted that cultures of specimens taken during revisions of failed shoulder arthroplasties are more likely to be positive for male patients than for female patients[2-4], our study provides the first evidence that the average bacterial load (as reflected by Shoulder Propi Scores and Average Shoulder Propi Scores) is almost 3 times higher in men than in women with at least 1 positive culture of a specimen taken during shoulder revision.

The Shoulder Propi Scores and Average Shoulder Propi Scores for the shoulders for which at least 1 culture was positive for Propionibacterium varied without a clear threshold above which a true-positive result could be defined with confidence.

These results need to be viewed in light of certain limitations. These retrospective data representing one of several possible approaches to specimen harvest are from the practice of an individual surgeon and the microbiology laboratory at one institution and thus the results of this study may not be generalizable. Although our sampling protocol has become more standardized over time, there was some variability in the number and type of specimens submitted for each revision case as well as variability in the sample size among the different sample types and among the samples of the same type. We also did not validate the Specimen Propi Score by comparing it with a validated quantitative measure of the number of bacteria present. We currently have yet to investigate the relationship of these culture metrics and treatment protocol to the clinical outcomes of revision shoulder arthroplasty.

In conclusion, we presented a method for standardizing the collection and reporting of culture data obtained at the time of revision shoulder arthroplasty. The Specimen Propi Values provide a means of demonstrating the differences in results among different types of specimens submitted for culture. They showed that Propionibacterium is not evenly distributed through a culture-positive shoulder. The Shoulder Propi Score and Average Shoulder Propi Score provide a means for characterizing the load of Propionibacterium in a revised shoulder and showed highly significant differences between men and women. The metrics used in this study demonstrate a continuous range of variability among patients, without evidence of a threshold that would clearly distinguish shoulders that are truly infected. We believe that, rather than viewing a specimen culture result as positive or negative, there is value in noting the degree of positivity as a reflection of the bacterial burden in the specimen and in the shoulder. Attention to the degree of positivity, or bacterial load, may provide clinically useful information about the importance of culture results in the evaluation and management of failed shoulder arthroplasty. As is the case for all laboratory tests, culture results need to be considered in the context of all other information available about the patient and the shoulder. ■

Zahab S. Ahsan, MD[1]
Jeremy S. Somerson, MD[2]
Frederick A. Matsen III, MD[1]

[1]Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, Washington

[2]University of Texas Medical Branch, Galveston, Texas

E-mail address for Z.S. Ahsan: zahsan@uw.edu
E-mail address for J.S. Somerson: jeremysomerson@gmail.com
E-mail address for F.A. Matsen III: matsen@uw.edu

References

1. Hou C, Gupta A, Chen M, Matsen FA 3rd. How do revised shoulders that are culture positive for Propionibacterium differ from those that are not? J Shoulder Elbow Surg. 2015 Sep;24(9):1427-32. Epub 2015 Feb 25.

2. Matsen FA 3rd, Butler-Wu S, Carofino BC, Jette JL, Bertelsen A, Bumgarner R. Origin of propionibacterium in surgical wounds and evidence-based approach for culturing propionibacterium from surgical sites. J Bone Joint Surg Am. 2013 Dec 4;95(23):e1811-7.

3. McGoldrick E, McElvany MD, Butler-Wu S, Pottinger PS, Matsen FA 3rd. Substantial cultures of Propionibacterium can be found in apparently aseptic shoulders revised three years or more after the index arthroplasty. J Shoulder Elbow Surg. 2015 Jan;24(1):31-5. Epub 2014 Jul 9.

4. Pottinger P, Butler-Wu S, Neradilek MB, Merritt A, Bertelsen A, Jette JL, Warme WJ, Matsen FA 3rd. Prognostic factors for bacterial cultures positive for Propionibacterium acnes and other organisms in a large series of revision shoulder arthroplasties performed for stiffness, pain, or loosening. J Bone Joint Surg Am. 2012 Nov 21;94(22):2075-83.

5. Updegrove GF, Armstrong AD, Kim HM. Preoperative and intraoperative infection workup in apparently aseptic revision shoulder arthroplasty. J Shoulder Elbow Surg. 2015 Mar;24(3):491-500. Epub 2014 Dec 2.

6. Foruria AM, Fox TJ, Sperling JW, Cofield RH. Clinical meaning of unexpected positive cultures (UPC) in revision shoulder arthroplasty. J Shoulder Elbow Surg. 2013 May;22(5):620-7. Epub 2012 Sep 13.

7. Frangiamore SJ, Saleh A, Grosso MJ, Alolabi B, Bauer TW, Iannotti JP, Ricchetti ET. Early versus late culture growth of Propionibacterium acnes in revision shoulder arthroplasty. J Bone Joint Surg Am. 2015 Jul 15;97(14):1149-58.

8. Frangiamore SJ, Saleh A, Grosso MJ, Kovac MF, Higuera CA, Iannotti JP, Ricchetti ET. α-Defensin as a predictor of periprosthetic shoulder infection. J Shoulder Elbow Surg. 2015 Jul;24(7):1021-7. Epub 2015 Feb 8.

9. Frangiamore SJ, Saleh A, Kovac MF, Grosso MJ, Zhang X, Bauer TW, Daly TM, Ricchetti ET, Iannotti JP. Synovial fluid interleukin-6 as a predictor of periprosthetic shoulder infection. J Bone Joint Surg Am. 2015 Jan 7;97(1):63-70.

10. Hudek R, Sommer F, Kerwat M, Abdelkawi AF, Loos F, Gohlke F. Propionibacterium acnes in shoulder surgery: true infection, contamination, or commensal of the deep tissue? J Shoulder Elbow Surg. 2014 Dec;23(12):1763-71. Epub 2014 Aug 29.

11. Mook WR, Klement MR, Green CL, Hazen KC, Garrigues GE. The incidence of Propionibacterium acnes in open shoulder surgery: A controlled diagnostic study. J Bone Joint Surg Am. 2015 Jun 17;97(12):957-63.

12. Sethi PM, Sabetta JR, Stuek SJ, Horine SV, Vadasdi KB, Greene RT, Cunningham JG, Miller SR. Presence of Propionibacterium acnes in primary shoulder arthroscopy: results of aspiration and tissue cultures. J Shoulder Elbow Surg. 2015 May;24(5):796-803. Epub 2014 Dec 4.

13. Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, Garvin KL, Mont MA, Wongworawat MD, Zalavras CG. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res. 2011 Nov;469(11):2992-4.

14. Butler-Wu SM, Burns EM, Pottinger PS, Magaret AS, Rakeman JL, Matsen FA 3rd, Cookson BT. Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. J Clin Microbiol. 2011 Jul;49(7):2490-5. Epub 2011 May 4.

15. College of Physicians & Surgeons of Sasktatchewan Laboratory Quality Assurance Program. Procedures/guidelines for the microbiology laboratory. 2010. http://ssu.ac.ir/cms/fileadmin/user_upload/Daneshkadaha/pezeshki/microb/file_amuzeshi/Lab_QA_Microbiology_QA.pdf. Accessed 2016 May 28.

16. Butler-Wu SM, Sengupta DJ, Kittichotirat W, Matsen FA 3rd, Bumgarner RE. Genome sequence of a novel species, Propionibacterium humerusii. J Bacteriol. 2011 Jul;193(14):3678. Epub 2011 May 13.

17. Grosso MJ, Sabesan VJ, Ho JC, Ricchetti ET, Iannotti JP. Reinfection rates after 1-stage revision shoulder arthroplasty for patients with unexpected positive intraoperative cultures. J Shoulder Elbow Surg. 2012 Jun;21(6):754-8. Epub 2012 Feb 3.

18. Horneff JG 3rd, Hsu JE, Voleti PB, O'Donnell J, Huffman GR. Propionibacterium acnes infection in shoulder arthroscopy patients with postoperative pain. J Shoulder Elbow Surg. 2015 Jun;24(6):838-43.

19. Kim SJ, Kim JH. Unexpected positive cultures including isolation of Propionibacterium acnes in revision shoulder arthroplasty. Chin Med J (Engl). 2014;127(22):3975-9.

20. Mook WR, Garrigues GE. Diagnosis and management of periprosthetic shoulder infections. J Bone Joint Surg Am. 2014 Jun 4;96(11):956-65. Epub 2014 Jun 4.

21. Warme WJ, Hsu JE. Definition of a "true" periprosthetic shoulder infection still eludes us. J Bone Joint Surg Am. 2015 Jul 15;97(14):e56.