Protocol

1. **Project Title:**
   How does Hydrogen Peroxide Application to the Wound Following Surgical Incision Affect C. Acnes Cultures in Primary Shoulder Arthroplasty?

2. **Investigators:**
   Thomas Wright, MD
   Kevin Farmer, MD
   Joseph King, MD
   Jonathan Wright, MD
   Alaina Ritter, MD

3. **Abstract:**

Cutibacterium acnes has been the focus of much recent shoulder literature, as it has been found to be both a common cause of periprosthetic joint infection as well as a common contaminant in shoulder surgery. Standard skin preparations have been found to be ineffective at eradicating *C. acnes* colonization on the skin and deep dermis of patients undergoing surgery. Recent literature has shown that skin preparation with 3% hydrogen peroxide solution is effective for decreasing the rate of cultures positive for *C. acnes* in both dermal and deep cultures; however, a positive rate of 10%-17% has still been reported despite this skin preparation. The current theory is that standard skin preparation does not remove *C. acnes* from the deep dermis which subsequently contaminates the deep tissue. We hypothesize that application of 3% hydrogen peroxide to the deep dermal layer immediately following the skin incision will even further decrease the rate of *C. acnes* contamination during surgery.

4. **Background and Significance:**

Over 50,000 people in the United States have shoulder replacement surgery each year. Of these, it is reported that 0.4%-2.9% of anatomic total shoulder arthroplasties (aTSA) and 1-10% of reverse shoulder arthroplasties (rTSA) are complicated by periprosthetic joint infection (PJI).[1,2]

Cutibacterium Acnes (*C. acnes*), an indolent organism found on the skin and in the sebaceous glands around the shoulder and back, makes the diagnosis of shoulder PJI particularly challenging. Multiple studies have shown that *C. acnes* can be isolated from deep cultures in up to 40% of patients without prior surgery undergoing primary total shoulder arthroplasty,[3] although whether this indicates actual colonization of the joint or contamination is an area

of debate. Regardless of the source of the bacteria, the fact that it was found in the joint prior to implantation of a shoulder arthroplasty is concerning from the viewpoint of PJI risk. This is particularly a problem in male patients, as studies have shown a sexual dimorphism with *C. acnes* cultures, with males having a 14-fold increased risk of being colonized by the bacteria.[4,5] Several centers have evaluated the possibility of the bacteria being present due to contamination from the skin edges, and it has been shown that standard skin preparation is unable to clear C. acnes from the skin in up to 50% of patients[6] and is even less successful at clearing the organism from the deep dermis, with up to 73% of dermal swabs from male patients immediately following the incision culturing positive for C. acnes.[5,7]

A recent development in shoulder surgery is the use of hydrogen peroxide to decrease the *C. acnes* load in the deep dermis sebaceous glands, with the hope that this will prevent contamination of the joint. Application of a widely commercially-available 3% topical Hydrogen peroxide in water solution *in vitro* has been found to completely eradicate *C. acnes* growth within 5 minutes[8] and has been shown in clinical trials when used as part of the skin preparation to decrease the rate of positive cultures for *C. acnes* from 35% to 10% for deep cultures[9] and from 34% to 17% for superficial dermal cultures.[10] While these results show marked improvement compared to standard skin preparations, the 17% with positive dermal cultures is still concerning given the significant morbidity associated with a PJI.

Our hypothesis is that an additional application of hydrogen peroxide to the dermis itself, immediately following the skin incision, will be even more effective at eradicating this potential source of contamination deep in the joint.

5. Specific Aims:

Specific Aim 1: Evaluate whether the hydrogen peroxide (H2O2) applied directly to the dermal tissue after the skin incision decreases the rate of dermal swabs positive for C. acnes.

Summary: Following our standard protocols for skin preparation (which includes an application of 3% H2O2, alcohol, and then Chloraprep in sequence) and draping (with Ioban applied over the skin), we will start the procedure as normal. The skin incision will be made in standard fashion, and the knife used will be removed from the surgical field. For the experimental cohort, a lap sponge soaked in 3% H2O2 will be applied to the incision and allowed to sit for 3 minutes. Following the 3 minutes, the sponge will be removed, the wound will be flushed with 100 mL of normal saline, and the exposed dermis will be swabbed and sent for culture. In the control cohort, the dermis will be swabbed and sent for culture immediately after the skin incision is made and the knife is removed from the field.

Hypothesis: We hypothesize that the control cohort will show results similarly to that published in the literature, with 35-70% of the cultures positive whereas the experimental cohort will have a significantly decreased rate of positivity, close to the negative control rate, likely less than 10%.

Specific Aim 2: Determine if H2O2 applied to the dermal tissue after skin incision will also decreases the rate of deep cultures of capsular tissue that are positive for C. acnes.

Summary: Following the procedure summarized for specific aim 1, the surgery will be carried out in a standard fashion in both cohorts. Bone, capsule, and labral tissue from around the glenoid that is routinely excised during the surgical procedure will be collected and sent for culture as described below.

Hypothesis: If the H2O2 is successful at decreasing the amount of contamination present at the deep dermal tissue, the rate of deep cultures positive for *C. acnes* will also decrease, supporting the hypothesis that unexpected positive deep cultures in primary shoulder arthroplasty are likely associated with contamination from the skin edge rather than due to colonization of the arthritic joint.

6. Research Plan:

Subjects: Once IRB approval has been obtained, up to 50 sequential male patients seen in the Hand & Upper Extremity and Sports Divisions in the Department of Orthopaedics & Rehabilitation who meet inclusion/exclusion criteria and who are boarded for primary anatomic or reverse total shoulder arthroplasty will be recruited into the study.

Inclusion Criteria:

- Male patients, age 40-90
- Undergoing primary anatomic or reverse total shoulder arthroplasty for glenohumeral osteoarthritis or rotator cuff arthropathy

Exclusion Criteria:

- Prior arthroscopic or open shoulder surgery on the ipsilateral shoulder
- Prior diagnosis of septic shoulder of the ipsilateral shoulder
- Corticosteroid injection within 3 months of the procedure
- Recently on antibiotics (within 2 weeks) prior to surgery

Assuming a rate of dermal cultures positive for *C. acnes* in the control group of 60% and a false positive rate of 10%, power analysis calculations were performed and resulted in a sample size of 13 patients per cohort needed to obtain a $\beta = 0.2$ and an $\alpha = 0.05$, for a total of **26 patients**.

Data Collection: Under an approved HIPAA waver, potential subjects will have their medical record and our clinical database reviewed by a member of the study staff. The following data points and information be collected for later analysis:

- Name

- Medical Record Number
- Date of Birth (to determine age)
- Gender (to determine eligibility)
- Diagnoses
- Shoulder surgical history
- Date of surgery
- Laterality
- Number of previous corticosteroid injections into the enrolled shoulder
- Past medical history of an acne breakout on the back/shoulders
- History of using prescription medications to treat acne
- Current anticoagulant or immunomodulatory medications

Additional data will be obtained during and after surgery:

- Type of implant placed (anatomic or reverse shoulder arthroplasty)
- Length of surgery
- Surgical complications
- Notes of difficulty obtaining the specified cultures
- Notes of any need to extend the skin incision during the procedure
- Postoperative wound complications

Methods:

Prior to surgery, enrolled subjects will be asked to complete a short medical history questionnaire. Subjects will then be randomized 1:1 into *Experimental* or *Control* cohorts. Randomization order will be determined by using a commercial random number generator that will be made into a log that will track group assignments. Subjects will added to the randomization log once they have received their final surgical clearance and the surgery order is determined for their surgery date. The result of the randomization will be reported by a member of the study team to the surgeon on the day of surgery prior to the start of the first procedure.

Following the standard protocol for skin preparation in shoulder arthroplasty surgery which includes an application of 3% H2O2 with gauze, isopropyl rubbing alcohol 70% USP with gauze, and then Chloraprep in sequence, and draping with Ioban applied over the skin, the procedure will start as normal.

The skin incision will be made in standard fashion, and the knife used will be removed from the surgical field. Next, for the *Experimental* cohort, a lap sponge soaked in 3% H2O2 will be applied to the incision and allowed to sit for 3 minutes. Following the 3 minutes, the sponge will be removed, the wound will be flushed with 100 mL of normal saline, any brisk bleeding will be controlled with electrocautery, and then the exposed dermis will then be swabbed and sent for culture. In the *Control* cohort, the dermis will be swabbed and sent for culture after the skin incision is made, the knife is removed from the field, and any brisk bleeding is controlled with electrocautery.

Each subject will have the following cultures taken during the procedure, using clean instruments for each culture:

1. Negative control: Before draping the patient, a culture swab will be opened on the back table and placed in the culture tube. This is essential to evaluate the false positivity rate of our lab, as other studies have reported rates of sterile, negative control cultures having a 9% positivity rate.[11]
2. Superficial culture #1: Following control of brisk bleeding with electrocautery as noted above, the exposed dermis will be swabbed (starting at the apex of the incision and going along the entire exposed dermis in a circular pattern twice).
3. Deep culture #1: Osteophytes are routinely removed from the humeral head after dislocation of the joint. These will be crushed and sent for culture.
4. Deep culture #2: After exposure of the glenoid, the stump of the long head of the biceps tendon is routinely excised with a portion of the labrum. We will do this in the normal fashion, and this tissue will be sent for culture.
5. Deep culture #3: Posterior and inferior labral tissue is also routinely excised and will be sent for culture following routine excision in the standard fashion.
6. Superficial culture #2: After the deltopectoral interval has been closed in standard fashion, the exposed dermis will again be swabbed as noted above.

All cultures will be sent to the lab blinded, with only the subject number and above-noted culture numbers reported to the lab rather than the location of each culture to avoid any potential bias regarding deep cultures and the negative control. We will order the standard 14-day OR hardware culture (aerobic and anaerobic) that has been recently set up at the University of Florida, which follows the recommendations of the consensus definition for periprosthetic shoulder infection published in 2019.[2]

Data Analysis:

Descriptive statistics will be used to describe both the experimental and control cohort's demographic characteristics. The two cohorts' demographics and culture results will then be compared for differences with use of standard statistical tests appropriate for the data and study results. The Student T-test will be used for continuous variables and the Fisher Exact test will be used for the categorical variables. Univariate analysis will be performed between the two groups given the small number of patients in each group and the randomized design of this study.

Confidentiality:

Once all subject data is collected and verified, subject PHI will be removed from the data sheet and the data will be maintained by using assigned research identification numbers that uniquely identify each individual. A separate key code will be kept until the end of the study. Dates will also be converted to time frames. Coded data will be stored in an Excel file on a password-protected encrypted server located in the Department of Orthopaedics and

Rehabilitation. Data analysis will also be stored on this server with only the study team members having access to these files. Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. After the study is completed, the key code will be destroyed and local data will be stored with other completed research studies in a secured storage room until the appropriate time when the research files may be purged according to university policy.

5. Possible Discomforts and Risks:

The only additional risk is the possible effect that application of H2O2 could have on wound healing. While some studies have shown that low-dose application of H2O2 to wounds can improve healing, there is some concern that the 3% H2O2 that will be used in this study is a high enough concentration that it can be detrimental to healing. This risk will be mitigated by rinsing away the residual H2O2 after applying it for 3 minutes to the deep dermis. Additionally, we consider this risk to be low as we often apply 3% H2O2 to surgical sites during primary and revision shoulder arthroplasty procedures after implantation of the components already to decrease the *C. acnes* bacterial burden within the joint on a case-by-case basis.

There will be no additional risk factors from culture techniques, and tissue that is routinely removed and discarded will be sent as the intraoperative deep cultures.

Data Safety & Monitoring Plan:

A Data and Safety Monitoring Plan (DSMP) will be implemented to ensure the safety of all participants involved in the study and to ensure the validity and integrity of the data. The Principal Investigator, with the advice and assistance of the study team, will monitor all aspects of safety. As part of standard of care, all patients undergoing shoulder arthroplasty receive wound education as part of their discharge instructions. Patients also receive continuous monitoring of their wound at post-operative Day 1 and at each clinic visit. As part of the safety monitoring program for this study, subjects will have their wound evaluated for 6 weeks post-operatively to both assure real-time participant care and unbiased monitoring of adverse outcomes. Signs of delayed wound healing, including drainage, erythema, induration, and wound dehiscence will be documented by the study team.

The Study Coordinator will meet weekly with the PI to review all events related to the study. Reports of the event will include a description of the event, a classification of seriousness, assessment of potential relationship to the study, assessment of need for change in the consent or the study activities, a summary of known prior health issues, event outcome and a classification of the main organ system involved. The classification of potential relationship to the intervention is as follows:

- **Definitely** - Temporal pattern + Known or expected AE response pattern

- **Possibly** - Temporal pattern + Known or expected AE response pattern + Could not have been produced by a number of other factors
- **Unlikely** – No Temporal pattern + Known or no AE response pattern + Could have been produced by a number of other factors
- **Definitely Not** - AE for which sufficient information exists to indicate that the cause is unrelated to the study intervention

Serious Adverse Events (SAE), as defined by the FDA, will be reviewed to determine their expectedness and relatedness to the study procedures. Any SAE's that are unexpected and related will be promptly reported to the IRB according to the guidelines outlined in the UF IRB-01 "Reporting – Adverse Events, Unanticipated Problems Involving Risks to Subjects or Others, Protocol Deviations, and Other Problems". All other events, including non-serious events, will be tracked and monitored using the logs provided by the IRB. A summary of these non-serious events will be reported to the PI and IRB. All events will be assessed and monitored by the study team until their conclusion.

6. Possible Benefits:

There will not be a benefit to study participants assigned to the *Control* group; however, those assigned to the *Experimental* group could see a benefit. Our hypothesized benefit is that by eliminating *C. acnes* from the deep dermis sebaceous glands, we can lower the incidence of *C. acnes* being seeded into the joint during the arthroplasty procedure, and, therefore, decrease the risk of developing a periprosthetic joint infection.

7. Conflict of Interest:

None

8. References:

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