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Anaerobic Thioglycolate Broth Culture for Recovery of *Propionibacterium acnes* from Shoulder Tissue and Fluid Specimens

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Propionibacterium acnes is an agent of shoulder infection, especially postsurgically (1, 2). The study by Butler-Wu et al. suggests a minimum culture incubation of 13 days for recovery of *P. acnes* from periprosthetic tissues and fluids (3). Methods for recovery of anaerobes vary, and parameters beyond duration of incubation may influence optimal *P. acnes* recovery. Butler-Wu et al. did not use anaerobic broth culture, and specimens were not transported under anaerobic conditions. We have routinely collected specimens for anaerobic culture into anaerobic tissue/fluid vials and have included an anaerobic thioglycolate broth incubated for 7 days in our anaerobic bacterial isolation strategy for tissue specimens. We had not been aware of any obvious limitation in isolation of *P. acnes* using this approach. In response to the findings by Butler-Wu et al., we examined whether 2-week culture incubation is needed for isolation of *P. acnes* when utilizing an anaerobic thioglycolate broth in conjunction with collection of specimens into anaerobic tissue/fluid vials.

Our study was performed over ~6 months in 2011. Immediately following collection in the operating room, orthopedic fluids and tissues were placed into anaerobic fluid vials (20-ml serum stopper vials with 1.3 ml prereduced peptone yeast extract broth, 0.5 g/liter cysteine hydrochloride, and 1 mg/liter resazurin indicator) or tissue vials (sterile 30-ml screw-top vials filled with CO_2), respectively. Tissues were homogenized using a Seward Stomacher 80 Biomaster (Seward Inc., Port St. Lucie, FL) in 3 ml of brain heart infusion broth for 1 min. A 0.1-ml sample of tissue homogenate or fluid in anaerobic transport medium was inoculated onto CDC anaerobic sheep blood agar and placed in a CO_2-flushed holding jar which, within 2 h, was set up with an AnaeroPack (Thermo Fisher Scientific, Lenexa, KS). After the jar was opened for plate examination, subsequent incubation was in a glove box at 37°C for 14 days. A 1-ml sample of fluid or homogenate was also inoculated into an anaerobically prereduced hemin-thioglycolate broth, which was closed and incubated at 37°C for 14 days. The broth was examined daily or until positive; cloudy broth was subcultured. The plate was examined Monday, Wednesday, and Friday for the first week and then on days 7 and 14 or until positive. Subjects with more than one specimen cultured on the same date (event) from a shoulder bone/joint source and with more than one specimen yielding *P. acnes* (1, 4) were analyzed.

Fourteen subjects had more than one positive shoulder bone/joint culture for *P. acnes*. (One subject had two events, and another subject, who had blastomycosis of the joint from which *P. acnes* was isolated, was excluded, yielding 14 analyzed events.) The Butler-Wu study was similar in size, with 17 patient events involving more than one specimen culture positive for *P. acnes* (3). Among the 14 events in our study, there were 72 anaerobic plate and broth cultures performed (range, 3 to 13 per event); *P. acnes* grew on 28 plates (27 within 7 days) and in 53 broths (52 within 7 days). All events had ≥2 positive broth cultures by 7 days, but three had negative plate cultures and another three had single positive plate cultures even with 14 days of incubation. No events were plate positive only. Broth was more likely to be positive than plate culture by day 7 and overall ($P < 0.0001$; chi-square test).

Some clinical practices, including ours, collect and transport specimens for anaerobic culture under anaerobic conditions; there has been no formal comparative evaluation of this approach to transportation under aerobic conditions (5). A myriad of solid and liquid anaerobic culture media are used to culture bone and joint specimens, but again, to the best of our knowledge, no formal comparison has been performed (5). Butler-Wu et al. used brucella agar inoculated with homogenized tissue using a sterile loop (3), a different solid medium, and possibly a smaller cultured specimen volume than used herein. Some investigators process anaerobic cultures (i.e., inoculate media) under anaerobic conditions (6), which was not done in the Butler-Wu study or in ours (3). Time to detection of *P. acnes* may depend on how frequently cultures are examined (e.g., daily, three times a week), anaerobic specimen collection/transportation and/or processing, volume of specimen cultured, and/or specific media used for culture. Our data support a 7-day incubation for anaerobic culture of *P. acnes* from bone and joint sites when using anaerobic thioglycolate broth with specimen collection into and transportation using anaerobic tissue and fluid vials. This approach does not require a specialized incubation environment.

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