Title:
Contaminant, colonization, or infection? Cutibacterium-specific assays to the rescue.

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Abstract:
Introduction. *Cutibacterium acnes* is the major pathogen associated with a shoulder periprosthetic joint infection (PJI), yet there is no assay available that can differentiate a true *C. acnes* infection from a skin contaminant. *C. acnes* dominates human sebaceous skin microbial communities, and various studies show high rates of false positive *C. acnes* cultures from intra-operative shoulder specimens. Distinguishing contaminant from true *C. acnes* PJI remains a significant clinical dilemma. **Strategies to quickly identify true *C. acnes* infections are desperately needed.** A biomarker of *C. acnes* growth could be used to address this problem. *C. acnes* protein RoxP is required for *C. acnes* to colonize human skin, only found in the Cutibacterium genus, and secreted by *C. acnes* both *in vitro* and *in vivo*. **We hypothesize that RoxP can be used as a *Cutibacterium* growth biomarker to identify true *C. acnes* PJI.**

Material & Methods. To determine if RoxP could be used as a *Cutibacterium* growth biomarker, we assessed it computationally; expressed it recombinantly; used generated four murine, monoclonal, anti-RoxP antibodies; developed RoxP immunoassays; and then assessed the specificity of these assays against specific RoxP orthologs.

Results. Structure-based sequence analysis of twenty-one RoxP orthologs (71-100% identity to *C. acnes* strain KPA171202 RoxP_1) revealed a high-degree of molecular surface conservation (**Figure 1a**), which suggested that an immunoassay could be developed that would identify the majority of *Cutibacterium*. The specificity of our anti-RoxP antibodies was assessed using ten recombinantly-expressed RoxP orthologs (71-100% identity, **Figure 1b**), and those results were used to develop an anti-RoxP immunoassay with nanogram sensitivity in three human biofluids (serum, synovial fluid, cerebrospinal fluid; **Figure 1c**). This immunoassay can detect 68% of all RoxP sequences, 71% of *C. acnes*-specific RoxP sequences, and 92% of *C. acnes* PJI strains.

Discussion. The assays developed in this work can now be used to assess RoxP's potential as a biomarker of *C. acnes* growth during human infection. We hypothesize that the detection of RoxP in human biofluids from sterile sites can differentiate true *C. acnes* infections from skin contaminants. Further, these immunoassays can be adapted to a point-of-care assay, which would be able to provide rapid, cost-effective, and sensitive detection of *C. acnes* infections in the outpatient setting. We believe that this *Cutibacterium* biomarker approach can significantly improve patient outcomes and decrease healthcare costs.

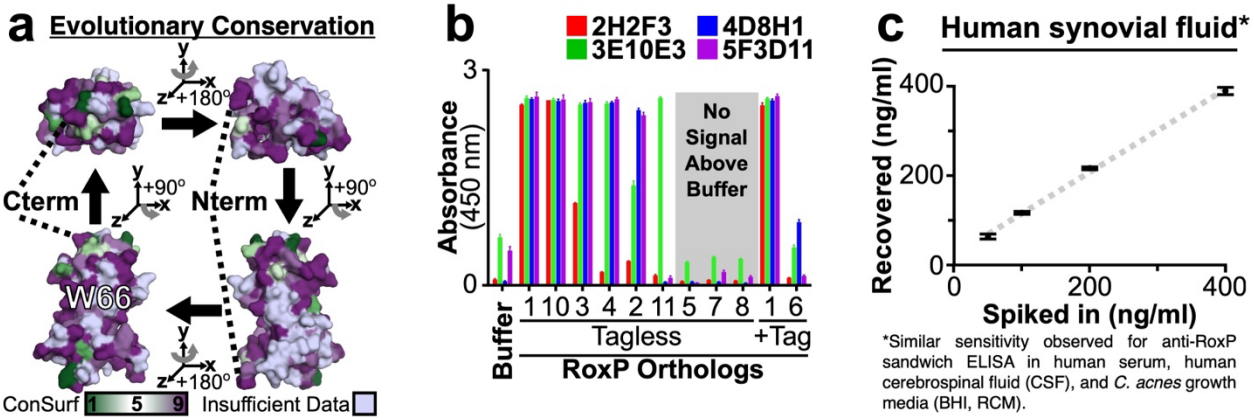

Figure 1. Development of a Cutibacterium-specific immunoassays. (**a**) Evolutionary conservation of RoxP across 21 orthologs. (**b**) Anti-RoxP antibody specificity assessed via indirect enzyme linked immunosorbent assay (ELISA) and a panel of 10 RoxP orthologs. (**c**) Example of RoxP sandwich ELISA sensitivity in a human biofluid.