Dear Editor,

We have read with interest the paper by Fritzell et al. which suggests the association of bacteria, especially the anaerobic bacterium *Cutibacterium acnes* (previously *Propionibacterium acnes*), with pain-generating degenerated discs is likely to reflect contamination arising from the skin. We find this view surprising given that the recent studies of Capoor et al. [1] and Ohrt-Nissen et al. [2] directly visualized *C. acnes* as a biofilm within surgically removed intervertebral disc tissue. Such observations are practically impossible to explain by contamination as this would require the contaminant to form a biofilm deep within a retrieved nucleus tissue fragment during the brief time between removal and freezing. Against this background, we would like to highlight a series of potential methodological limitations within the Fritzell et al. study that could impact on their final results and conclusions regarding the association of *C. acnes* with degenerated discs.

**Sample collection**

Although *C. acnes* is aerotolerant, when using culture as a detection method it is still best practice to collect samples in a manner similar to that used for strict anaerobes, and as exemplified for detection of *C. acnes* in prosthetic joint samples [3]. Such an approach will help to ensure maximum recovery of the bacterium within disc samples that have counts in the lower range (10^2–10^3 CFU/g) [1]. Ideally, samples should also be immediately transferred to the laboratory under an anaerobic atmosphere and processed within an anaerobic cabinet. Any diluents and liquid media used for sample transport and processing should also contain a suitable reducing agent like l-cysteine hydrochloride (0.05% w/v) and have been pre-equilibrated under anaerobic conditions.

**Biofilm disruption and broth enrichment**

In such a study, it is important to identify the type and quantity of organisms associated with the samples at the time of analysis. Were the organisms on the surface of the sample or imbedded within the sample? No details were given to understand how the samples were processed once they arrived at the clinical laboratory. It would appear that biopsy samples were directly used to inoculate agar plates and also placed in enrichment broth for culture, although how the culture results collected for each patient relate to these different methods is not described. Critically, there is no indication that biopsy samples were processed by
Molecular detection

This study also used broad-range 16S rDNA sequencing to examine disc and vertebral samples, with largely negative results. It is, however, unclear how well the primers used match and, therefore, react with the different ribotypes of C. acnes, and what their detection limit cut-off (CFU/g tissue) is. Although the authors assert that their assay is a routine diagnostic, no evidence of any validation in respect to positive and negative controls, the absence of PCR inhibitors, etc., was provided. The method for DNA isolation is also not described, and as C. acnes has a thick cell wall, protocols using a bead-beater or similar method to enhance cell lysis and DNA yield from small bacterial numbers, as well as biofilm, should have been followed [4].

Genetic relatedness

As highlighted earlier, while contamination of spinal surgery wounds can be expected in a proportion of cases, WGS sequencing of only one or a few isolates cultured from a disc or vertebra biopsy sample and matching skin or soft tissue is not, in our opinion, sufficient to absolutely confirm this in individual cases. Multiple clones will likely coexist on the skin surface, so a comprehensive mapping of multiple C. acnes phylotype signatures from both sources is required for robust conclusions to be made. We believe that such WGS experiments, in the absence of tissue homogenization prior to culture, or ideally a biofilm detection method, such as fluorescence in situ hybridization coupled with confocal laser scanning microscopy, have minimal value since they tell us little about any bacteria that is embedded within the disc sample, and not optimally detected.

Understanding the role that C. acnes plays in DDD has been complicated due to its ubiquitous presence on the skin and its potential to contaminate samples during spinal surgery. Nevertheless, the observation of C. acnes as a biofilm within disc tissue, combined with animal studies, which have demonstrated the capacity of the bacterium to induce disc degeneration and inflammatory responses in the end plate region, suggests a plausible causal role as a pathogenic factor in DDD. We believe that the best methodological approach for future studies investigating the role of C. acnes in DDD is to utilize disc tissue homogenization combined with imaging techniques that are sensitive to the presence of biofilms, but eliminate the issue of contamination. With such an approach, one is less likely to throw the proverb “baby out with the bathwater”.

Compliance with ethical standards

Conflict of interest MNC, OS, CB, AM, FA, AR, KM and VAF have stock ownership or options in DiscitisDx, Inc. MNC and OS have filed several patent applications, which have been assigned to DiscitisDx, Inc.

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