## A Comparative Methodology: Processing Laryngotracheal Swabs for 16S rRNA Gene Sequencing

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The microbiome of a "healthy" vocal fold in a human model has yet to be characterized in the relatively new field of otolaryngology. The use of bacterial identification as a comparison of healthy versus normal, in the future, may prove to be an effective tool for exploring treatment options in vocal fold injury and disease patients. For more current work regarding what is a "healthy" and "unhealthy" vocal fold microbiome, an identified processing method for low-biomass swabs using next-generation 16S rRNA gene sequencing could give researchers an alternative to tissue biopsies and bacterial cell cultures. A biopsy of the true vocal folds is problematic because it can lead to scarring and dysphonia, and therefore cannot ethically be used to evaluate healthy human vocal folds. Tissue biopsy methods for microbial sequencing limits researchers to animal studies, which may not even be indicative of accurate results in humans. In bacterial cell cultures, swabs can be taken and streaked onto various agar plates to culture bacteria. However, since bacterial growth is very specific as to time, temperature, and medium, some of the bacteria originally present in the sample may not eventually culture. This means the results are not accurate, and they may not provide a complete representation of bacterial species present at the time of swab collection. The importance of this work comes in as a reliable, accurate, and efficient alternative to vocal fold tissue biopsies and bacterial cell culture by directly processing low-biomass swabs for the purpose of vocal fold microbial identification.