



Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

Online Proceedings

POSTER SESSION 2

Commons West, Easel 8

1:00 PM to 2:30 PM

Analysis of Oral Swabs for Universal Bacterial 16s rDNA to Optimize Diagnostic Application

Divya Naidoo, Senior, Public Health-Global Health

Mentor: Gerard Cangelosi, Environmental and Occupational Health Sciences

Mentor: Rachel Wood, Department of Environmental & Occupational Health Sciences

Mentor: Alaina Olson, Environmental and Occupational Health

Oral swab analysis (OSA) is a possible alternative sample type for tuberculosis diagnostics. It has been observed that tongue swabs contain greater amounts of *Mycobacterium tuberculosis* DNA than cheek swabs ($p < 0.0001$) from tuberculosis patients. After determining that oral microbiota follows this same pattern, several factors including time-of-day swabbed and health status were analyzed to understand factors affecting the amount of bacteria on the tongue. This project aims to optimize the oral swab sampling methods in order to facilitate more sensitive diagnostic tests, using universal bacterial 16s rDNA as a proxy for *Mtb* DNA. Previously tested samples from South Africa were further analyzed to investigate amount of oral microbiota by day collected, HIV status, health status, and other demographic factors. To evaluate whether collecting multiple swabs per sample yielded more universal bacterial DNA, tongue swabs were taken from healthy volunteers in Seattle. Each subject provided a 1-swab sample and a 3-swab sample, which was then extracted and analyzed by a previously optimized universal bacterial PCR. Additionally, tongue scrapers are being assessed as an alternative to oral swabs. Swabs collected early in the morning had more bacterial DNA than swabs collected later ($p < 0.03$). 3-swab samples yielded an average of 2-fold greater amounts of bacterial DNA than 1-swab samples. Bacterial biomass correlated with *M. tuberculosis* signal in most comparisons. Bacterial biomass may serve as a useful proxy when developing better oral swab sampling strategies for TB diagnosis.

POSTER SESSION 4

Commons West, Easel 4

4:00 PM to 6:00 PM

Optimizing Oral Swab Analysis for Tuberculosis Diagnosis

Rita Noor Olson, Senior, Microbiology

Mentor: Rachel Wood, Department of Environmental & Occupational Health Sciences

Mentor: Gerard Cangelosi, Environmental and Occupational Health Sciences

Tuberculosis (TB) remains a major international health concern and one of the top 10 causes of death worldwide, according to the World Health Organization. Previous clinical work in our lab demonstrated that oral swab analysis (OSA) can successfully diagnose tuberculosis by detecting *Mycobacterium tuberculosis* DNA in the mouths of infected patients. In order to strengthen OSA against traditional but more invasive methods, such as sputum sampling, improvements to DNA extraction and swab type must be investigated. I am comparing different degrees of automation with Mol-Bio's Trueprep, the AudioLyse, and ThermoFisher's King-Fisher against our previously validated manual Qiagen extraction protocol—with the comparison lying in sensitivity and efficiency. In order to increase the versatility and sensitivity of OSA, I am also investigating boil preparations and dissolvable swabs. Boil preparation of swabs is a relatively simple extraction procedure, and early results have demonstrated its comparability against the Qiagen extraction. Meanwhile, dissolvable swabs have a hypothetical 100% yield of sample material. I have successfully dissolved calcium alginate swabs from Puritan in acidic sodium citrate solutions, and Luna swabs were dissolved in chaotropic agents. DNA yields are compared to non-dissolvable, previously validated swabs.