No Cough Required
Tongue swabs for the diagnosis of tuberculosis with Cepheid Xpert® MTB/RIF Ultra

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Introduction

Tuberculosis (TB) remains a leading cause of infectious disease morbidity and mortality with an estimated 1.4 million deaths in 2019. Sputum (phlegm coughed from the respiratory system) is the most collected diagnostic specimen for TB diagnosis. The Cepheid Xpert MTB/RIF and 2nd generation Ultra are the most widely used automated nucleic acid amplification tests (NAAT) for diagnosis of TB. Sputum has drawbacks. Sputum is difficult for many to produce (especially children). Sputum production is also a potentially dangerous procedure when infection prevention controls are not in place. Tongue swabs can be easily and quickly collected with minimal risk. Tongue swabs using manual qPCR methods have acceptable sensitivity when compared to other diagnostic methods. Studies looking at tongue swab samples with Ultra have exhibited suboptimal results. My work screened ~30 tongue swab storage and extraction methods and characterized 3 promising methods, comparing them to the qPCR method.

Methods

Swabbing
- TB-negative participants self-swab with a COPAN FLOQSwab for 10-15s firmly pressing along the tongue. Samples are then spiked with MTB.

qPCR Method
- Uses a commercial Qiagen DNA extraction kit with ethanol precipitation.

Xpert Ultra Methods
- Method 1: 1 swab boiled for 10 minutes. 2 volumes of storage buffer added. Incubated and shaken.
- Method 2: 1 swab. 2 volumes of Cepheid's inactivation Sample Reagent (SR) added. Incubated and shaken.
- Method 3: 2 swabs. Same processing as Method 2.

Discussion, Next Steps

Method 3 is suitable for clinical evaluations. Constant balance between error rate and sensitivity, while still ensuring sample rendered non-infectious. Although Method 1 is the most sensitive method, it suffers from a high over-pressurization error rate. These errors arise when the internal cartridge membrane become blocked/clogged. The addition of SR in Methods 2 and 3 greatly reduced the error rate, although there was a decrease in sensitivity. Method 3 showed improved sensitivity compared to Method 2 and recent research has shown that only ~10% of biomass is removed with one swab (Wood et al., 2021, under review), highlighting that 2 swabs can potentially pick up more MTB and boost sensitivity. Clinical evaluations of Method 3 currently underway. Additional processing methods should continue to be evaluated.

Sputum-like buffers with SR. Passing sample through needle after boiling.

References: