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

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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 2019¹.

Sputum (phlegm coughed from the respiratory) system) is the most collected diagnostic specimen for TB diagnosis¹.

> The Cepheid Xpert MTB/RIF and 2nd generation Ultra are the most widely used automated nucleic acid amplification tests (NAAT) for diagnosis of TB¹.

Sputum has drawbacks. Sputum is difficult for many to produce (especially children)². Sputum production is also a potentially dangerous procedure when infection prevention controls are not in place³.

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^{4,5}.

Studies looking at tongue swab samples with Ultra have exhibited suboptimal results^{6,7}.

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 precipitation⁴.

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.

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Method 3 is suitable for clinical evaluations.

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

> The addition of SR in Methods 2 and 3 greatly reduced the error rate, although there was a decrease

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

- Passing sample through needle after boiling.

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