

## Optimize the detection of mixed Mycobacterium tuberculosis (MTB) infections

### **Introduction:**

Tuberculosis (TB) is an infectious contagious disease transmitted through droplet nuclei particles formed by the evaporation of coughed or sneezed airborne particles containing (*Mtb*), *Mycobacterium tuberculosis* bacteria. Transmitted through respiratory routes from person to person, tuberculosis commonly affects the lungs, but can damage any tissue, making it the world's leading causes of ill-health and the first cause of death from infectious disease (1). Each year 10 million people fall ill with tuberculosis (TB), despite being preventable and curable disease, 1.5 million people die from TB each year (2). Leading TB countries, such as South Africa, are often challenged by low socioeconomic status of a settings and limited resources, treating TB as a continuous uniform epidemic(3).

Concepts such as mixed strains infection phenomenon pose a major challenges in controlling tuberculosis (TB). However, advances in molecular methods such as genome sequencing (WGS) promise the role of genomic epidemiology, uses natural variability in *M. tuberculosis* complex DNA as a tracking tool, and is used to identify individual risk factors of TB (4). This is important as TB epidemics are comprised of multiple simultaneous chains of transmission that could be isolated simultaneously distincting targets and revealing more details about mixed infection with high resolution.

A growing body of evidence suggests that geographically targeted interventions may be effective and cost-efficient in high-burden, low-resource settings, and could be instrumental in accelerating progress toward TB elimination(4). Thus understanding epidemiology and clinical impact of mixed TB infections,with the concept of mixed infections. The impact of TB transmission and clinical outcomes,can be optimized with the role of genomic epidemiology,detection methods.

**Purpose/Objective:**

The overall goal is to optimize the detection of mixed Mycobacterium tuberculosis (MTB) infections. Achieved by comparing different specimen sampling time and different approaches to culture MTB and extract mycobacteria DNA. Data will be based on the Enrollment TB patient time of three samples, and the sample type of 1) Direct Extraction 2) Early Growth 3) Full Culture, where sequencing of WGS and targeted amplicon deep sequence will be analyzed. Detection to the maximized heterogeneity will allow for a crucial step in improving the understanding of TB transmission in high TB incidence settings by accounting for within-individual heterogeneity of MTB infections.

**Previous and ongoing work:**

Previous work contribution involved conduct data management and ensure quality control involving data examination using data analysis softwares, R. This accounted for a visualized and statistical analysis while also testing hypotheses involving laboratory data. Using data analysis, antibody response/development overtime within students was compared between three categories: 1) no prior COVID-19 diagnosis; 2) prior COVID-19 diagnosis; and 3) SARS-CoV-2 vaccinated students via antibody measurements.

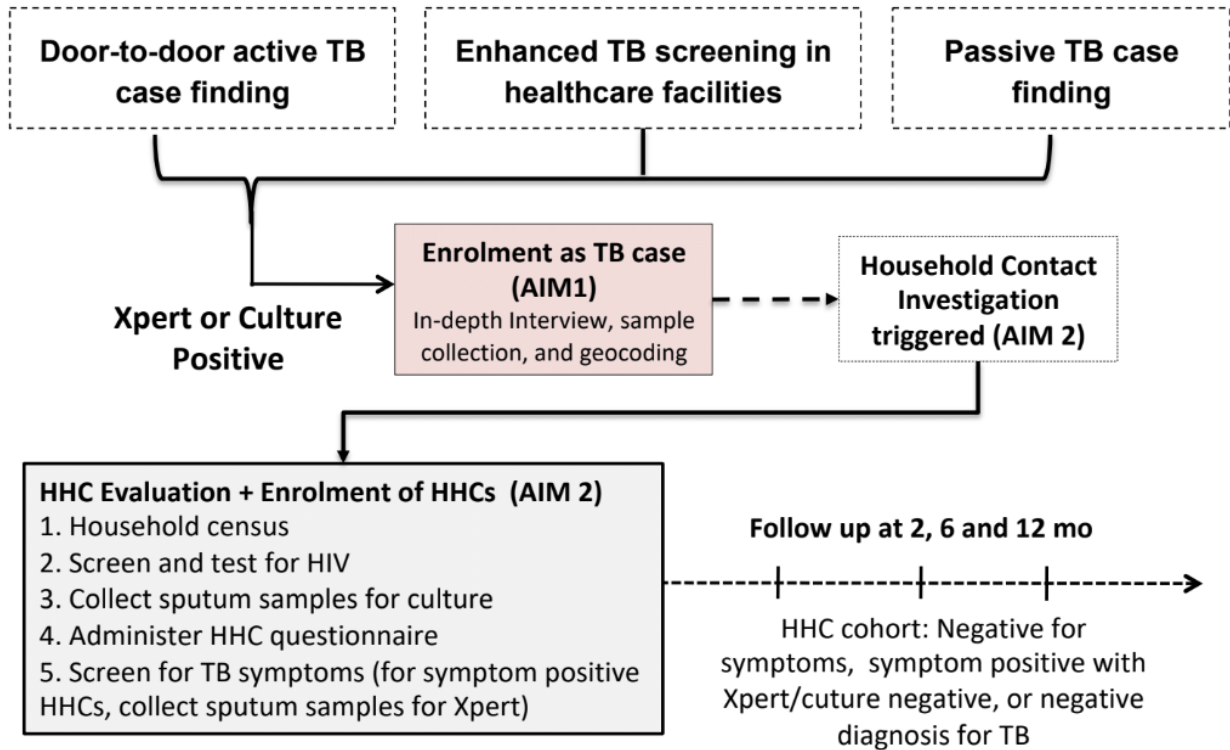
This prospective cohort study involved a longitudinal serial collection of antibody measurements from blood samples via ELISA. Students completed an online RedCap study screen for informed consent. Results showed that the first dose of Moderna developed relatively higher concentrations of antibodies compared to Pfizer-BioNTech ( $P= 0.067$ ) while for the second dose had no significant difference ( $P= 0.92$ ). The same method and strategies will be applied to data on TB.

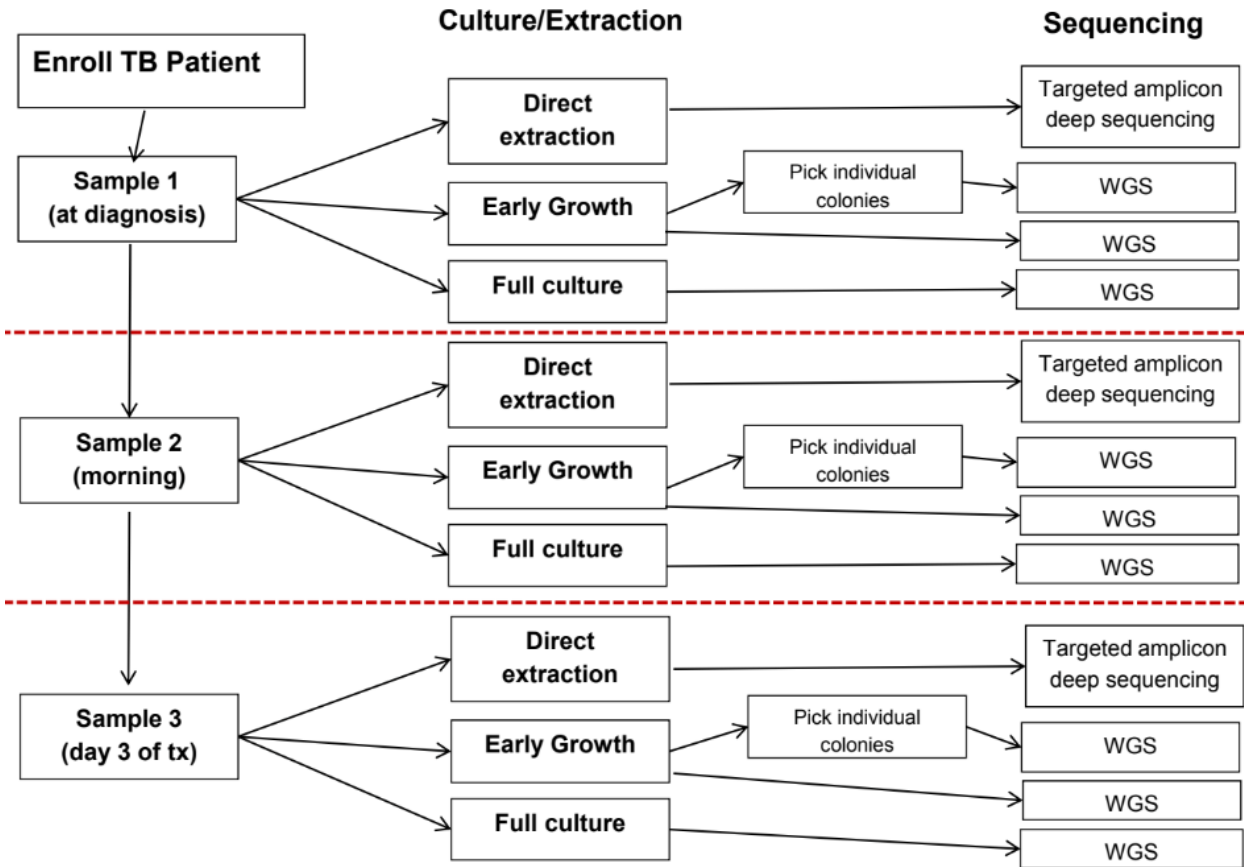
**Methods:**

Methods have been conducted with enroll patients from participating healthcare clinics in Greater Gaborone, Botswana. All persons who screen positive for TB symptoms are considered presumptive TB cases and will undergo microbiological evaluation by Xpert as per Botswana National TB Program guidelines. Those who have laboratory-confirmed TB will be included in this pilot study.

Collect 2 sputum samples at 3 different time points from each enrolled TB patient. Next, we will extract MTB DNA by different methods at the pre-specified time points. Extracted MTB DNA will be shipped to Borstel, Germany for analysis by targeted deep sequencing and whole genome sequencing (WGS). This document describes the active TB screening procedure and pilot experimental protocol.

Method of Parent study will be applies to data collected.





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**Student Responsibility:**

Student will help with data analysis and from the gathered three samples dividing them into direct extraction, early growth and full cultures of TB disease.. For the SURP project, I will conduct data management and ensure quality control involving data examination using R software as data is collected, real-time. This will be done via computer algorithms that identify possible errors and inconsistencies in the data measurements. My contribution during the

summer will be. Compare the level of heterge in the 3 culture/extraction and the enroll TB patient, during culture , day one.

Using this data I will perform data analysis related to tuberculosis development in sequencing overtime by comparing the difference between TB exposure. I will maintain contact with my research team, including the PIs and my UCI faculty mentor, regularly through email and weekly video conferences.

**Timeline:**

June-August 2020	Continue training in statistical analysis of laboratory data. Performing data cleaning and data quality control procedures.
August – September 2020	Data analysis and report writing
September 15, 2020	Completed project

**Budget:**

Stipend of \$3000 is requested

**Reference: (change it)**

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- (2) WHO (World Health Organazation) Global Tuberculosis Report 2022 [Internet]. [cited 2022 May 2]. Available from: [https://www.who.int/health-topics/tuberculosis#tab=tab\\_1](https://www.who.int/health-topics/tuberculosis#tab=tab_1)

- (3) Narasimhan, Padmanesan et al. “Risk factors for tuberculosis.” *Pulmonary medicine* vol. 2013 (2013): 828939. doi:10.1155/2013/828939  
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- (4) Chelsea R. Baker, Ivan Barilar, Leonardo S. de Araujo, Anne W. Rimoin, Daniel M. Parker, Rosanna Boyd, James L. Tobias, Patrick K. Moonan, Eleanor S. Click, Alyssa Finlay, John E. Oeltmann, Vladimir N. Minin, Chawangwa Modongo, Nicola M. Zetola, Stefan Niemann, Sanghyuk S. Shin. “High-resolution characterization of recent tuberculosis transmission in Botswana using geospatial and genomic data – the Kopanyo Study” medRxiv 2022.04.13.22273731; doi: <https://doi.org/10.1101/2022.04.13.22273731>
- (5) Votintseva AA, Pankhurst LJ, Anson LW, Morgan MR, Gascoyne-Binzi D, Walker TM, Quan TP, Wyllie DH, Elias CDO, Wilcox M, Walker AS, Peto TEA, Crook DW. Mycobacterial DNA Extraction for Whole-Genome Sequencing from Early Positive Liquid (MGIT) Cultures. *J. Clin. Microbiol.* 2015; 53: 1137–1143.