RAPID DETECTION OF TUBERCULOSIS USING DROPLET BASED MICROFLUIDICS
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ABSTRACT
Tuberculosis is one of the most deadly diseases that kills over one million people each year and infects one-third of the world’s population. Early diagnosis is critical to the prevention and control of TB. In this study a rapid method to detect indicator enzyme specific to M. tuberculosis is presented.

KEYWORDS: Tuberculosis, rapid detection, micro-fluidics.

INTRODUCTION
Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is a major global health problem. In 2011, there are approximately 8.8 million new active TB cases, and 1.1 million deaths [1]. Fortunately, TB is curable with a treatment success rate of 85% [2]. The biggest challenge is in the prompt diagnosis of TB: the World Health Organization (WHO) estimated TB detection rate to be just 63% [2]. Acid-fast smear using sputum is the front-line diagnostic test for TB, but it does not become positive until a few months after transmission occurs. Furthermore, its sensitivity varies from 20-80% [3]. Isolation and culture of Mtb is more sensitive, and remains the gold standard for TB diagnosis. Mtb culturing takes 4-8 weeks to become positive [4], however. Culturing facilities are also poorly available in developing countries. As such, there is an urgent unmet need for a simple, sensitive, and portable assay for the detection of active Mtb infection [4]. In this study we describe a method for the rapid detection of a reporter enzyme from Mycobacterium tuberculosis based on the isolation of bacteria in picoliter droplets. Our vision is that this tool will be able to detect Mtb in a sensitive, rapid, specific and quantitative manner. This method is expected to find use in resource-limited settings where TB is the most prevalent.

EXPERIMENTAL
A schematic description of a potential device for rapid detection of TB based on droplet microfluidic technique is depicted in Fig 1. The device consists of 3 parts: (i) Droplet generation and compartmentalization of the sample with probe. As droplets flow downstream in the microchannel, the content in the droplet is mixed rapidly. (ii) Incubation of the probe for fluorescence to turn on. (iii) Detection channel where fluorescence signal from the drops is interrogated.

We use BlaC, a β-lactamase naturally expressed by Mtb, as our reporter enzyme. Previously, we had designed a BlaC-specific fluorogenic substrates as a probe for Mtb (Fig 2) [5]. This fluorogenic substrate is chemically linked to a quencher. It does not fluoresce due to close proximity of the quencher. It becomes fluorescent after cleavage of the linker—the hydrolysis of the β-lactam, generating a fluorescent product whose emission is increased >200 folds (Figure 2ab). Since BlaC is surface-localized, the probe does not have to traverse bacterial cell wall in order to be cleaved, making our marker ideal for fast diagnosis of Mtb [5]. Since this probe is a catalytic reporter, our method can be very sensitive because it is not limited by number of tagged molecules that can be delivered to the cell. Instead, the fluorescent probe can be continuously produced to increase the signal as long as substrate is available.
RESULTS and DISCUSSION

In our initial experiments, we used *E. coli* expressing BlaC for characterization. We spiked a sample with known concentrations of bacteria (10^4–10^6 cfu/mL). Using a microfluidic flow-focusing nozzle, the sample was mixed with the fluorogenic substrate and then emulsified into droplets of various volumes. The emulsion was incubated at room temperature and the fluorescence intensity from the drops was monitored at various time points. Figure 3a shows that fluorescence signal increased faster in smaller drops. This is expected as the effective concentration of a single bacterium in a smaller volume is higher than that in a larger volume, thereby accelerating the enzymatic reaction. Using 30-μm drops, the signal reached saturation in 150
minutes, orders of magnitude faster than the current standard. Figure 3b shows a 1:1 correlation between the detected bacteria count versus the input bacteria concentration. Current work is in progress to characterize the lowest detection limit of our method.

![Image of Figure 3](image.png)

**Fig 3:** (a) Normalized intensity as a function of incubation time for various droplet sizes and for the bulk. (b) Comparison between the concentration measured from the fluorescent drops and the concentration measured using NanoDrop.

**CONCLUSIONS**

In this study we have developed a new method for rapid detection of tuberculosis using droplet-based microfluidics. This method is based on the isolation of tubercle bacillus in a large number of picoliter droplets combined with a fluorescent probe. We used BlaC (an enzyme naturally expressed/secreted by tubercle bacilli) as a marker and a designed BlaC-specific fluorogenic substrates as probe for Mtb detection. We have shown that isolating the bacteria and the probe in a large number of picoliter droplets can shorten the detection time significantly compared with existing methods. Our vision is that this tool will be able to detect tubercle bacilli in a sensitive, rapid, specific and quantitative manner, and will be useful in resource-limited settings where TB is the most prevalent.

**REFERENCES**


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