A platform for DNA extraction for paperfluidic diagnostic devices

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Project Summary: Many tests are currently used to confirm tuberculosis, including smear microscopy, pathogen culture, serological testing, nucleic acid amplification tests (PCR, for example), and others. The most rapid and affordable test currently available is smear microscopy, but it is not able to distinguish Mycobacterium tuberculosis from other mycobacteria and requires additional testing for confirmation. Despite taking up to four weeks, bacterial culture is sensitive and specific. The sensitivity of a diagnosis based on culture is not present in serological assays, although they are quick. Conversely, mycobacterium tuberculosis can be uniquely identified using nucleic acid amplification tests, which also have higher sensitivities than serological or microscopy diagnostic methods. Unlike MTB cultures, which could take up to several weeks, PCR gives results within a few hours. However, PCR requires the use of thermocyclers which are not always available in low resource settings. Therefore, we explored an enzymatic isothermal amplification technique (helicase dependent amplification or HDA, specifically) as an alternative to PCR since HDA can be carried out at a fixed temperature using any suitable heat source, e.g. a hot plate [fig.1].

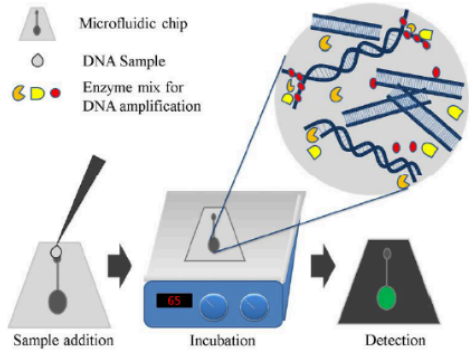

Fig.1. Schematic diagram of our proposed MTB DNA detection

In parallel, we investigated using paper as a substrate for DNA amplification in order to eliminate the requirement for costly microfluidic cartridges, which are presently a component of several of the commercial TB diagnostic platforms, including Gene Xpert (Cepheid Inc.). Paper can be printed into zones that are hydrophobic or hydrophilic, and it is inexpensive and plentiful. Without the use of an external pumping mechanism, fluid wicks through paper via capillary action. There is less possibility of contamination or infection spread from sputum samples because paper devices can be burned after a test. So far, our paperfluidic device has successfully worked with purified Mycobacterium tuberculosis DNA. However, the sample preparation steps (i.e. extraction and purification of DNA from the bacterial pathogen) for HDA are still carried out off-chip. For this technology to be useful in field applications, we need to be able to work with sputum samples taken from TB patients. The biggest obstacle to developing a point-of-care diagnostic system for actual field use is the integration of the sample preparation process, especially for tests involving nucleic acids. Almost all papers (close to 3000 published in the last decade) reporting on-chip DNA analysis rely on off-chip sample preparation and reagent handling. Currently there are only two commercially available nucleic acid analysis devices that have integrated an automated sample preparation step: GeneXpert (Cepheid) and Liat Analyzer (Roche). Hence, there is a big technology gap in integrating sample preparation steps with the on-chip DNA analysis systems that needs to be addressed. Therefore, in this proposal we aim to explore affordable DNA extraction techniques that can be integrated with our amplification platform.

Specific problem being addressed: The World Health Organization (WHO) estimates that 1.3 million individuals lost their lives to tuberculosis in 2012. Approximately 3 million patients did not receive a diagnosis out of the 8.6 million individuals who contracted tuberculosis in 2012. The diagnostic technologies that are now on the market are

often too expensive or unsuitable for settings with limited resources. It has been observed that TB and HIV frequently coexist. Furthermore, due to numerous drug resistance, treating tuberculosis presents additional difficulties after a diagnosis. Given the high prevalence of tuberculosis in India, this is an especially concerning scenario. Thus, there is a critical need for a reasonably priced tuberculosis diagnostic chip that meets the specifications of the intended product, which UNITAID, WHO describes as a "rapid, sputum-based, cartridge-based molecular test for microscopy centers." This proposal aims to address this problem.

Impact of this innovation: A 2014 UNITAID report states that the Gene Xpert MTB/RIF system's launch significantly altered the TB diagnostics industry, with over 7.5 million cartridges being sold at discounted costs in 108 nations. However, this platform is primarily used to test patients with combined TB and HIV infections and is generally not used for early detection of suspected TB cases. Most countries with high incidences of TB, such as India, still rely on sputum smear microscopy as the sole diagnostic test as it is affordable. This points to a strong unmet need for a “rapid, sputum-based, molecular test for microscopy centres”. Clearly, there is considerable economic potential for such a rapid, affordable, sensitive and specific diagnostic test in India. We expect our proposed technology to fit into this technology gap.