Point-of-Care Magnetic Barcode Assay for Genetic Pathogen Detection

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Statement of Purpose: Tuberculosis (TB) remains a public health threat and economic burden with an estimated worldwide prevalence at 14 million cases and 1.7 million deaths. Despite the high availability of treatment for positively diagnosed TB patients, the task of identifying those patients remains a challenge mainly because of inadequate technology platforms. Furthermore, the alarming emergence of drug-resistant *Mycobacterium tuberculosis* (MTB) has increased the need for a reliable TB detection platform. Thus, a tool that rapidly diagnoses MTB and identifies drug-resistant strains will reduce TB mortality, reduce the rate and spread of drug resistant acquisition, and be transformative for clinical care in resource-limited settings.[1] Here, we report on the development of a nucleic-acid platform designed for point-of-care TB detection. The platform can detect MTB down to the sensitivity of few hundred CFU/mL and also discriminate single-nucleotide polymorphism in rifampin-resistant strains directly from minimally processed samples within 2.5 hours.

Methods: The magnetic barcode assay for detecting MTB nucleic acids consists of three components: 1) DNA-modified capture beads (diameter: 1 µm), 2) amplicons from polymerase chain reaction (PCR), and 3) DNA-modified magnetic nanoprobes (MNPs, 30 nm). Following mechanical DNA extraction from the sputum samples, the target DNA region is amplified through PCR using MTB-specific primers. The amplicons were captured on the beads modified with complementary DNA strands. The beads were rendered magnetic by coupling the MNPs to the opposite end of the amplicons.[2] After removing the unbound MNPs, nuclear magnetic resonance (NMR) measurements were applied on the beads. The presence of MNPs led to a faster decay in the water protons' spins that could indirectly quantify the presence of target DNA. To automate the nucleic acid detection process, we integrated the PCR amplification, magnetic barcode assay, and NMR measurements into a single microfluidic device. Following PCR amplification, the reagents were mixed along an extended channel. The MNP-labeled beads were purified by an in-line membrane filter and concentrated into the miniaturized NMR (µNMR) chamber for detection. An automated feedback system on the device monitored and compensated for temperature drifts, and enabled consistent measurements across different temperature environments (4–50°C).[3]

Results: µNMR measurements of the magnetically labeled beads showed high signal in the presence of target DNA, but low nonspecific binding of the MNPs in presence of non-complementary strands. The sequence-specific hybridization between oligonucleotides enabled highly selective amplification of MNP-loading onto the bead surface. By applying a pair of MNPs conjugated with complementary sequences, we could form multiple MNP-layers and thus amplified particle loading onto beads. With PCR-amplification of genomic MTB DNA, the system could detect down to a few genomic DNA, demonstrating the potential to detect a single bacterium. The clinical utility of the system was evaluated by using sputum samples spiked with whole MTB to final MTB concentration from 0 to 10^7 CFU/mL. The measurements established that the detection limit was 10^2–10^3 CFU in 1 mL sputum. Signals from sputum samples containing 10^6 non-MTB bacteria were nearly identical to those from control sputa, confirming the specificity of the assay. We tested the developed platform to detect rifampin-resistant and wild-type strains of MTB in sputum samples. Extracted DNA samples were inserted into the device and amplified by PCR for *rpoB* (drug resistance) and *fadE15* (MTB detection) regions. While the wild-type strain yielded only positive signal for the *fadE15* probes, the mutant isolates yielded positive signals for both the *fadE15* probes as well as the corresponding mutant *rpoB* probes. The control samples containing non-MTB mixed bacteria yielded negative signals for both *rpoB* and *fadE15* probes.

Conclusions: An accurate, robust and inexpensive point-of-care diagnostic test is essential to achieve global control of MTB. The presented µNMR device delivers a portable diagnostic tool that can be translated to clinical settings. Unlike culture-based testing that can require 2-8 weeks, the nucleic acid µNMR platform offers simple readout for both MTB presence and antibiotic-resistance directly from mechanically-processed sputum samples within 2.5 hours. These features will not only reduce delays in patient treatment, but also decrease costs related to the drug-susceptibility testing. Combining all these features in the nucleic acid µNMR, an automated TB diagnostics will become accessible for clinical settings within TB endemic areas.

References: 1) Lee H. Angew Chem Int Ed. 2008;48:5657-5660. 2) Liong M. Adv Mater. 2011;23:H254-H257. 3) Liong M. Bioconjug Chem. 2011;22:2390-2394.

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