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Integrated Nucleic Acid Testing for TB diagnosis in Peripheral Settings: Sample Preparation, Amplification, and Detection Processes

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There is an urgent need for rapid diagnosis of tuberculosis (TB) accessible to affected populations in regions with high TB incidence, to facilitate effective treatment and limit disease transmission. We are developing an easy-to-use fully integrated nucleic acid testing device to enable TB diagnosis from sputum. This system automates pathogen lysis, nucleic acid extraction, isothermal DNA amplification and lateral flow detection. The sample preparation, amplification, and detection methods implemented in this device are present herein. To disinfect and liquefy a sputum sample, we developed a process using inexpensive and stable reagents, which reduces the viable *Mycobacterium tuberculosis* (*M.tb*) load in sputum ≥1e6 fold, thus mitigating biohazard concerns. Pathogen lysis and DNA extraction is performed in < 10 min inside the single-use, inexpensive, disposable PureLyse® miniature bead blender (Claremont BioSolutions), which effectively lyses mycobacteria via a battery operated micro-motor and impeller. PureLyse® sample preparation uses a novel solid-phase extraction method that does not require chaotropic salts or organic solvents. We demonstrated that the PureLyse® method provides comparable performance to a clinically validated reference sample preparation method, which requires 1.5h to complete. This comparison used quantitative PCR to analyze extracts obtained via both methods from human sputum spiked with *M.tb* cells, and from a small set (N = 45) of clinical sputum samples from TB suspects. Our device can implement different isothermal amplification methods. We currently use Loop Mediated Amplification (LAMP) targeting the *M.tb* gyrB gene, which we have coupled to nucleic acid lateral flow (NALF) detection. We developed a novel approach to enable stable mastermix reagent storage at elevated temperatures in a format suitable for integration into our device. In ongoing stability studies we found that the master-mix remains functional after storage for > 1 month at 25°C or 37°C. The DNA-containing PureLyse® eluate can be used directly to reconstitute the dry mastermix reagents, and the amplified mastermix can be applied directly to the NALF strip for detection, without the need for additional liquid reagents. The limit of detection for this dry reagent LAMP-NALF assay is currently ~ 100 copies *M.tb* DNA spiked into mock PureLyse® eluate. This assay can identify DNA extracted from phylogenetically diverse *M.tb* strains, without showing cross-reactivity with a set of Non-Tuberculous Mycobacteria. We further demonstrated that the entire assay process consisting of sputum disinfection and liquefaction, PureLyse® sample preparation, dry mastermix reconstitution and isothermal LAMP, followed by NALF, can detect 10^3 CFU *M.tb* per mL sputum, with further optimization ongoing. These processes are amenable for integration into a disposable cartridge approximately the size of a mobile phone, with an associated compact battery operated instrument, to enable molecular diagnosis of TB in near-patient low-resource settings of high-burden areas.