

## **Integrated Nucleic Acid Testing for TB diagnosis in Peripheral Settings: Cartridge and Instrument**

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There is an urgent need for methods that enable rapid and affordable diagnosis of tuberculosis (TB) accessible to affected populations in regions with high TB incidence, to facilitate effective treatment and limit disease transmission. We have developed an easy-to-use fully integrated nucleic acid testing device for TB diagnosis from sputum, consisting of a disposable cartridge approximately the size of a mobile phone, with an associated compact, inexpensive, battery operated instrument. This system automates pathogen lysis, nucleic acid extraction, isothermal DNA amplification and lateral flow detection. To date, we have demonstrated fully integrated process execution using sputum spiked with *Mycobacterium tuberculosis* H37Ra. This sample was liquefied, disinfected, and injected into the inlet port of the cartridge. Without further user intervention, a visual readout was obtained within 1.5 h from sample introduction. Inside the cartridge, sample preparation is performed in the SimplePrep<sup>TM</sup> valve (Claremont BioSolutions), a single-use, inexpensive, disposable six port active valve that integrates the PureLyse<sup>®</sup> miniature bead blender for cell disruption and DNA extraction. This design eliminates the need for multiple passive/active valves. As the sample passes through the valve into the waste chamber, liberated DNA is captured on beads inside the blender. Next, wash buffer and then elution buffer is pumped through the valve, to remove impurities, and to release purified nucleic acids from the beads. These pumping steps are facilitated through miniaturized and inexpensive electrolytic pumps (ePumps) integrated into the cartridge, which generate pressure based on water electrolysis. The DNA-containing eluate is pumped past a venting chamber to remove dead air, and into a reaction chamber, containing dry mastermix reagents. This reaction chamber entails an innovative double pouch design that enables effective heat transfer, and facilitates pumping, in conjunction with custom designed inexpensive check valves. After isothermal amplification inside the reaction chamber, the sample is pumped onto a lateral flow strip for visual detection. All required reagents are stored inside the cartridge in a thermo-stable form. The closed system cartridge mitigates amplicon carryover contamination. Automated process execution is facilitated by a compact inexpensive instrument. Upon cartridge insertion, the instrument performs initial checks to verify appropriate heater and valve operation, and to ensure that all electrical connections between cartridge and instrument are functional. Once all checks are passed, the user is prompted to inject the sample. During test execution, the LCD screen displays the process status, and generates error messages if a step fails. Upon successful completion, the system generates a message that the lateral flow strip can be read. This device will be significantly less expensive than currently available fully integrated bench-top systems for TB nucleic acid testing, and is designed using low power electronics to enable battery operation. It is suitable for use by minimally trained personnel and does not require additional laboratory equipment, regular maintenance, or temperature controlled facilities. Such a device can enable TB diagnosis and treatment initiation in the same clinical encounter in near-patient low-resource settings of high TB burden countries.