Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information:

Integrated Nucleic Acid Testing System to Enable TB Diagnosis in Peripheral Settings

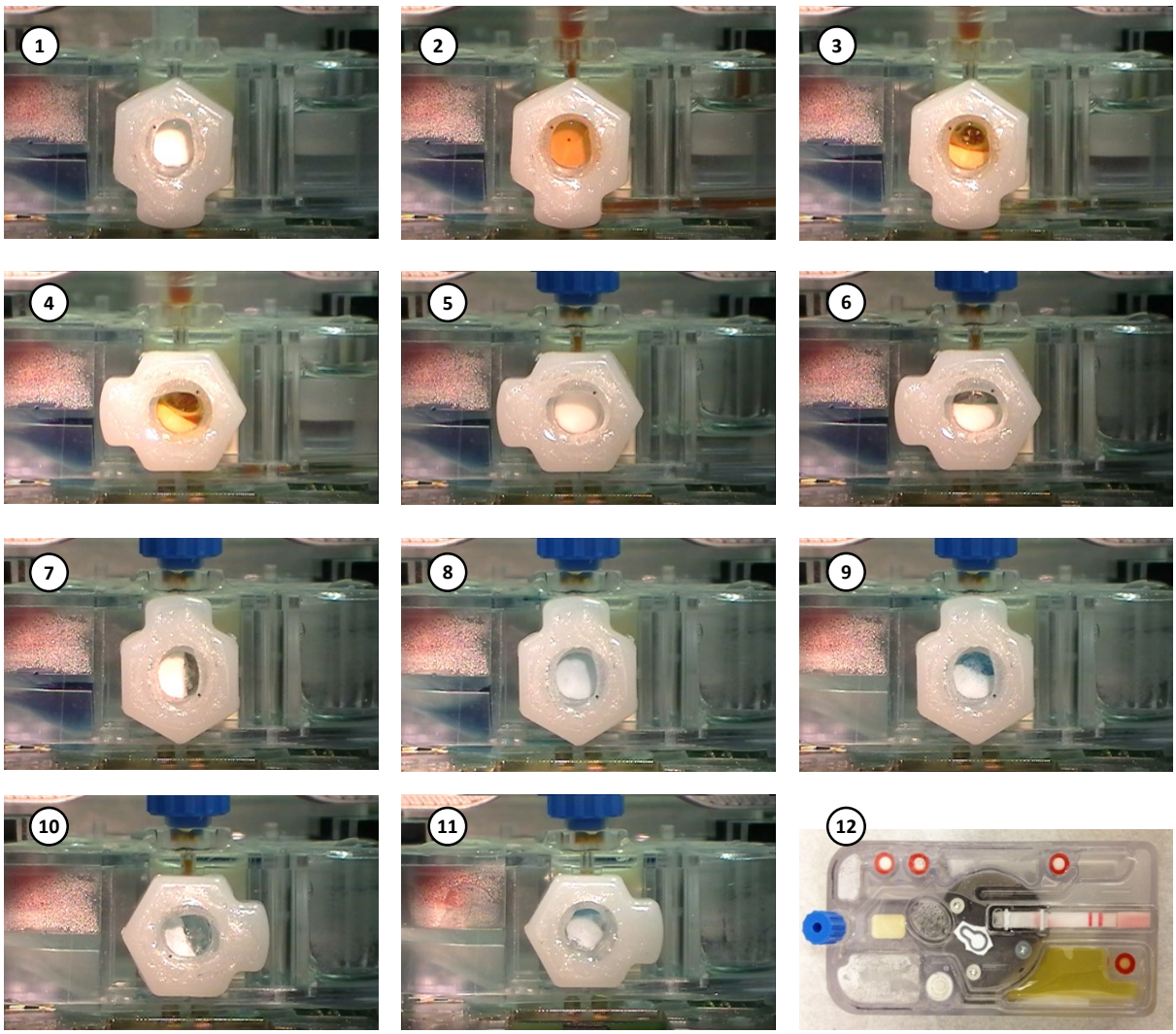

Figure S1. Process execution visualized in a cartridge with modified OmniValve that contains a transparent window. (1) Initial state with valve in the 0° position, with wash buffer chamber on the right (clear solution) and elution buffer chamber on the left (solution dyed blue for fluidic testing). (2) Liquefied and disinfected sputum sample (dyed yellow for fluidic testing) is injected through the OmniValve to the waste chamber, while the blender motor is activated at 6.5V, which lyses the bacteria and leads to DNA binding to the beads inside the OmniValve. (3) End of sample injection. (4) The OmniValve is turned to the 90° position, to connect the PureLyse® device with the wash and waste chambers. (5) Wash buffer is pumped through the OmniValve, with intermittent blender motor activation at 1.5V. (6) Wash is completed and ePump purges the OmiValve of wash buffer. (7) The OmniValve turns to the 180° position, to connect the elution chamber to the holding chamber #1. (8) Elution buffer fills the OminValve, and the cartridge incubates the beads with elution buffer to release the bound DNA. (9) All eluate is pumped to holding chamber #1. (10) The OmniValve turns to the 270° position to connect the holding chamber #1 to holding chamber #2. (1) All eluate is pumped into holding chamber #2 and from there into the reaction insert for isothermal amplification. (12) Following amplification, the amplified master-mix is pumped into the lateral flow strip chamber for detection.

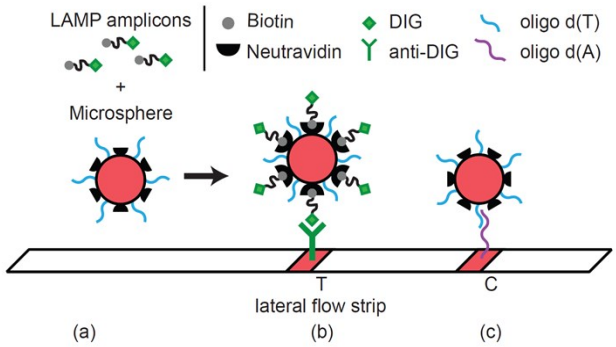

Figure S2. Concept of LAMP amplicon detection via NALF: (a) LAMP amplicons labelled with biotin and DIG bind coloured polystyrene microspheres functionalized with Neutravidin. (b) After migrating along the nitrocellulose membrane, microsphere-bound amplicons are captured at the test line via DIG-anti-DIG interaction. (c) At the control line, microspheres are captured irrespective of the presence of amplicon via interaction between oligo-d(A) and –d(T).

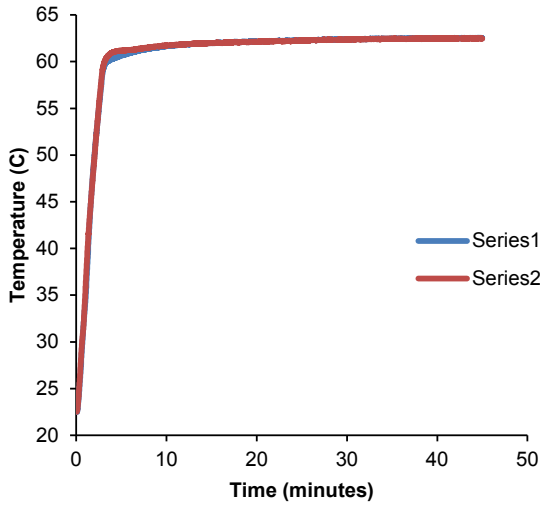

Figure S3. Thermal control of fluid within the reaction pouch in two independent runs performed in two different cartridges: initial ramp rate 12.2 °C/min. Starting from room temperature, the system reached 62±1°C within 6.1 min, with no overshot. The system maintained a temperature of 62.2±0.3°C for the duration of the run. This data was recorded using a thermocouple inserted into the reaction pouch of the cartridge filled with reaction buffer.

Table 1: Instrument Bill of Materials

Item	Price
Controller PCB + Components	$126.24
Connector PCB + Components	$57.72
Stepper motor components	$49.25
Heater Components	$37.64
Display and buttons	$23.87
12V Power Supply	$13.35
Structural Housing Components	$110.00
Internal Hardware	$45.00
Total	**$463.07**

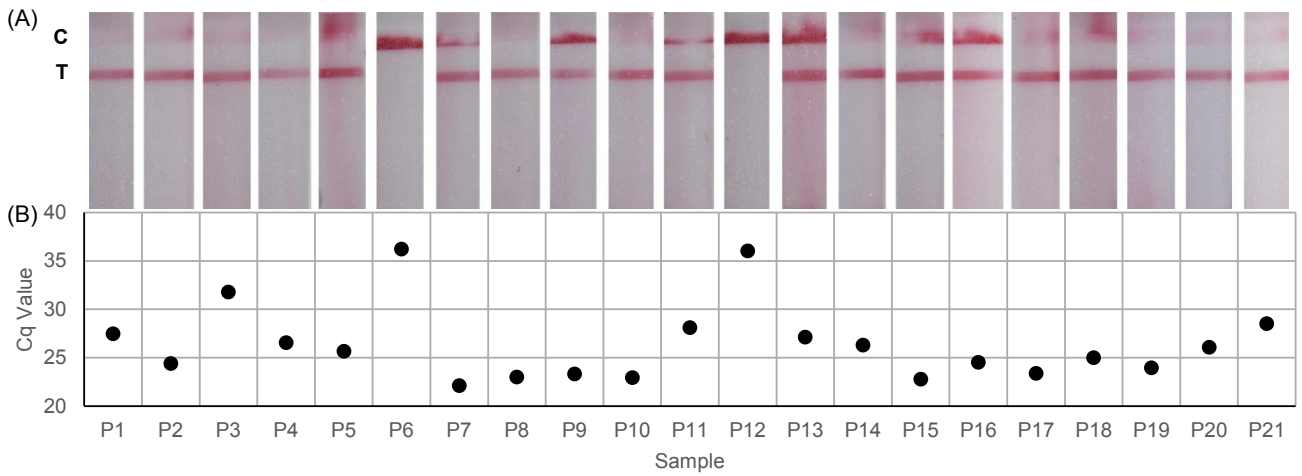

Figure S4. (A) Lateral flow strip images of the 21 *M.tb.* positive clinical sputum samples run in the cartridge by the instrument. T: Test line, C: Control Line. If a signal appears at the test line, the sample is considered positive, even if the control line does not show up. Samples P6 and P12 represent false negative results. (B) Cq values of the qPCR comparator method for each of the positive clinical samples. Samples with Cq values ≤37 are considered positive. The false negative samples P6 and P7 had Cq values of 36.2 and 36, respectively, thus are paucibacillary, i.e. contained a low *M.tb.* bacterial load.

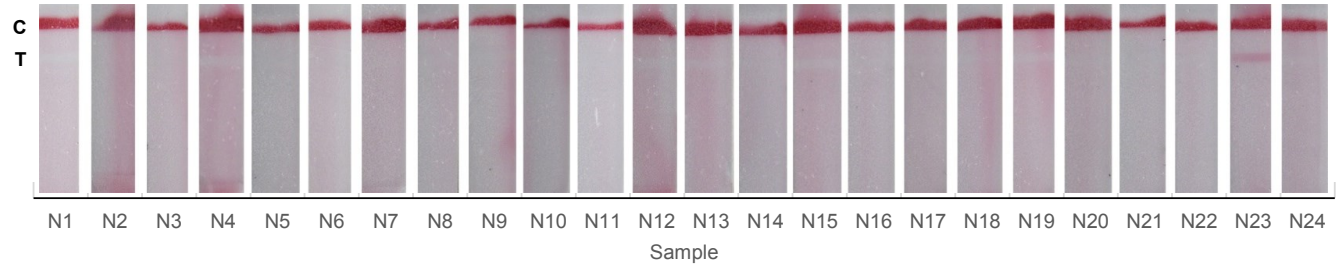

Figure S5. Lateral flow strip images of the 24 *M.tb.* negative clinical sputum samples run in the cartridge by the instrument. T: Test line, C: Control Line. If no signal appears at the test line but a signal appears at the control line, then the sample is considered negative. Sample N23 represents a false positive results. None of these samples gave an amplification signal in the qPCR comparator method, therefore there is not applicable Cq data for the negatives.

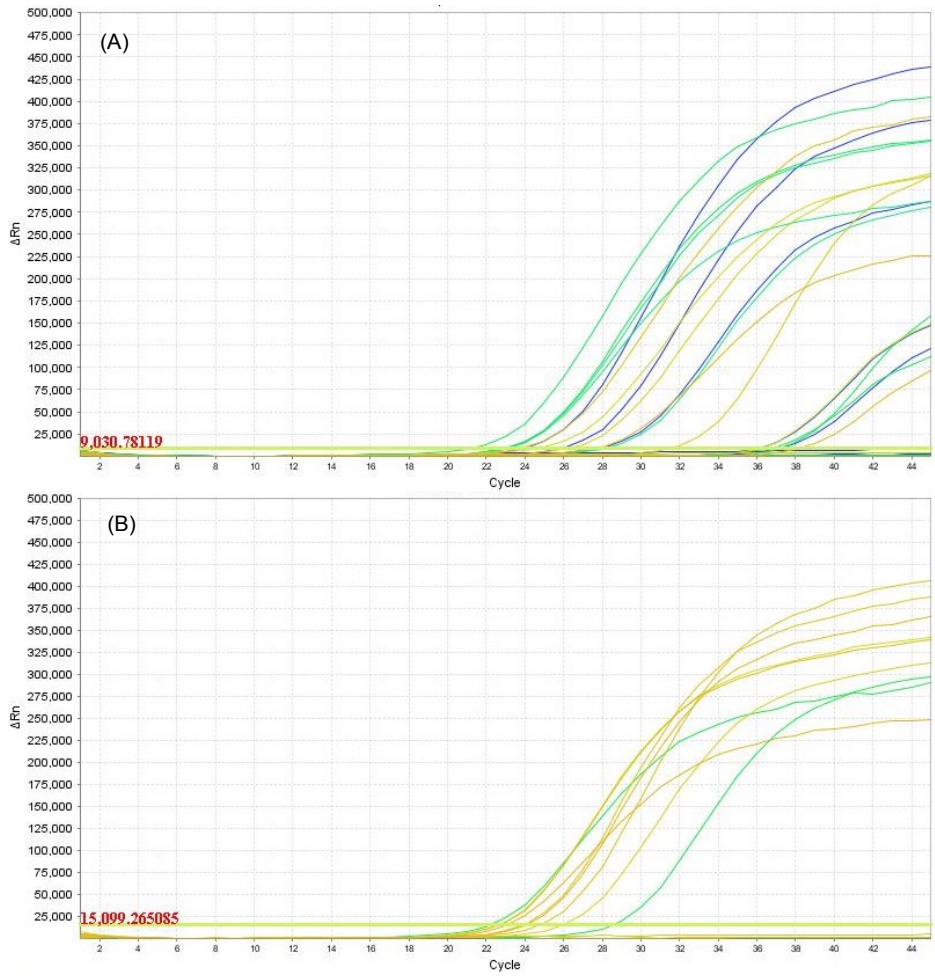

Figure S6. Comparator method qPCR data of 39 specimens run in duplicate, from patients who were diagnosed by microbiological culture and clinical criteria as active pulmonary TB cases, and who were put on treatment. (A) specimens 1-27, (B) specimens 28-39. Specimens were iteratively tested with the comparator method until 23 qPCR-positive samples had been identified. Samples were deemed qPCR-positive when both reactions yielded Cq values ≤37. For 16 specimens the qPCR result was negative, e.g. due to sample acquisition later on in the disease progression and after initiation of antibiotic therapy.

Figure S7. Comparator method qPCR analysis of 25 specimens run in duplicate, from patients who visited the clinic with suspected TB but were subsequently determined by microbiological and clinical criteria to not have TB, and were not put on treatment. (A) samples 1-15; (B) samples 16-25. All samples were qPCR negative.