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ON AN INTEGRATED, LOW-COST HEATING SYSTEM*

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# CENTRIFUGAL LABTUBE FOR FULLY AUTOMATED DNA EXTRACTION & LAMP AMPLIFICATION BASED ON AN INTEGRATED, LOW-COST HEATING SYSTEM

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## ABSTRACT

In this paper, we introduce a disposable battery-driven heating system for loop-mediated isothermal DNA amplification (LAMP) inside a centrifugally-driven DNA-extraction platform (LabTube). We demonstrate fully automated, fully closed extraction of as little as 100 DNA copies of verotoxin-producing (VTEC) *Escherichia coli* lysate in water, milk and apple juice in a standard laboratory centrifuge, followed by subsequent automatic LAMP amplification with an overall time-to-result of 1.5hrs. The system is disposable, fully closed and automated, requiring only a single pipetting step. The microcontroller-driven heating system is low-cost (<1\$) and it can be easily parallelized. Because the heated LabSystem runs within a standard laboratory centrifuge, it is suitable for DNA extraction and amplification in low-resource areas, at production sites or sales locations.

**KEYWORDS:** Automated DNA extraction, DNA amplification, LAMP, centrifuge

## INTRODUCTION

DNA extraction and amplification are key elements of DNA-based analysis. Currently, DNA extraction requires extensive handling and pipetting steps. To automate those processing workflows, the LabTube platform was recently introduced [1] (Figure 1A). The LabTube is a new microfluidic platform for Lab-on-a-Chip applications and it is based on modules integrated in a falcon tube. The sample and reagent processing workflow can be automated by applying process specific centrifugation protocols. In order to increase the extraction efficiency (e.g. pre-heating of lysis or wash buffers), quality (e.g. ethanol removal) and to incorporate downstream analysis methods (such as DNA amplification or immunoassays), a cost-efficient heating system is desirable. As a first application, the isolation and amplification of *E.coli* (VTEC) lysates was incorporated into the LabTube, yielding a fully-automated and contamination-free system that only requires a single pipetting step. VTEC *E.coli* produce Shiga-like toxin, a major source of foodborne illness. They are oftentimes found in contaminated water, meat, dairy products and juice. When infecting humans, they have been linked with the severe complication haemolytic uremic syndrome [2]. Since testing is often time-critical, rapid extraction methods and tests that can be used at the production site, in the field or at the point-of-care are desirable.

## EXPERIMENTAL

Verotoxin-producing *E.coli* lysate (EDL 933, purchased from Biotecon) was extracted using the Micro DNA kit (Qiagen, Cat. no. 56304). Loop-mediated isothermal DNA amplification (LAMP) of verotoxin-producing *E.coli* was performed using a commercial kit (Mast Diagnostica, Cat.no. 67vtsck3). Real-time PCR (Applied Biosystems) and LAMP reactions inside a thermoblock were always run in parallel as a control. The reaction products were confirmed using electrophoresis (Agilent 2001 Bioanalyzer). At least three runs were performed per experiment.

The LabTube battery encasings were designed in Solid Works and the microcontroller was programmed using Bascom. All runs inside the LabTube were performed in a Hermle swing-bucket centrifuge (Z326K).

## RESULTS AND DISCUSSION

As shown in Figure 1B, as little as  $10^2$  copies of VTEC *E.coli* DNA were purified inside the LabTube using the QIAamp Micro DNA kit, which yielded higher extraction efficiencies than other tested silica-column based kits. The extraction efficiency was  $157\pm 33\%$  compared with the manual reference. Since the desired test result does not have to be quantitative, an isothermal DNA amplification method, LAMP (Mast Diagnostica), was chosen to specifically detect the organism of interest. Unlike PCR, LAMP does not require thermal cycling. Instead it runs at a constant temperature of  $65^\circ\text{C}$ , which allows for a simpler and cheaper (disposable) heating system to be used.

The heating system was controlled and driven by a low-cost microcontroller (ATXMEGA) (Figure 2). As a power source, a 3V CR-2 battery was chosen, which was the only battery to physically fit into the LabTube (based on size and weight) and that at the same time yielded the required power (0.5W) for more than 1hr. The heating system in revolver

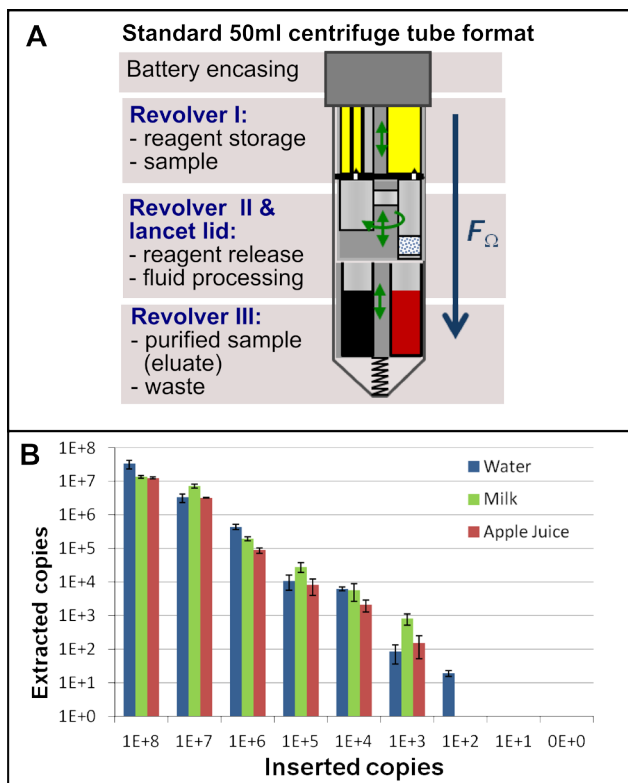


Figure 1. (A) Schematic of the LabTube components. (B) DNA extraction of VTEC *E.coli* (EDL 933) lysate in water, milk and apple juice inside the LabTube ( $n \geq 3$ ). Using the QIAamp Micro DNA kit, the extraction limit is  $10^2$  in water and  $10^3$  copies in milk and apple juice as shown with qPCR. The average extraction efficiency is  $157 \pm 33\%$  compared with the manual reference.

III was connected with the microcontroller through a cable. It consisted of two SMD resistors for heating and a NTC for temperature regulation. To achieve homogeneous temperatures, these elements were embedded in heat-conducting cement surrounding an aluminum-wrapped sample chamber. The components for the heating system are low-cost (<1\$) and the heated LabTube can therefore be used as a disposable platform. The achieved temperature profile was stable to  $\pm 2^\circ\text{K}$  (Figure 3), which is sufficient for LAMP amplification.

Mechanical and fluidic functionalities of the system were verified by processing the heated LabTube inside the centrifuge. In the standard protocol, pen mechanics, actuated by centrifugal forces, rotate revolver II by changing the centrifugal speed over time. This mechanism allows for opening and closing of fluidic paths through the stack and thus liquid routing. The mechanics of the system were successfully verified, since revolver II moved from the start (S) to end (E) position during the protocol whilst all components remained intact (Figure 4). For fluidics verification, revolver I was filled with water colors for fluid tracking. At the end of the run, the PCR tube had collected the desired amount of eluate (blue), whilst all the remaining liquids were transferred to the waste chamber (orange) (Figure 4).

The LAMP reagents were stored in the elution chamber and the amplification started immediately after the eluate was purged into the chamber. The reaction was visualized via a visual detection dye that changed its color upon the 40min reaction. As shown in Figure 5, the negative control stayed purple, whilst the positive control changed its color to dark blue. Moreover, quantitative electrophoresis confirmed the desired amplification product in the positive sample, whilst the negative control contained no product. The overall time-to-result for DNA isolation and amplification was 1.5hrs.

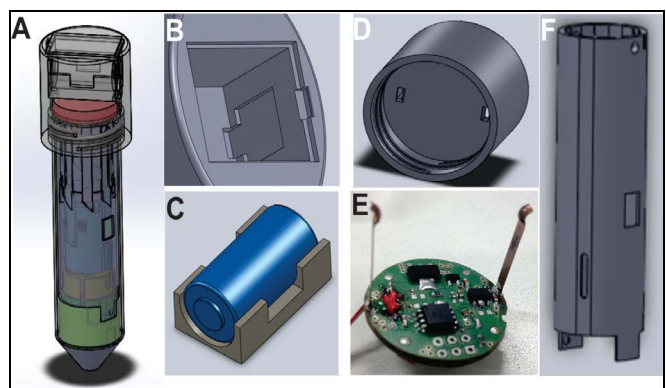


Figure 2. (A) Complete LabTube with battery encasing. (B) Cap with holes for electrical contacts of the microcontroller to contact the battery sitting above it. (C) The battery is embedded in a soft constricted piece. (D) It is sitting in a cavity. (E) Below it sits the round circuit board with the microcontroller. (F) LabTube encasing with a hole for cables. The cable runs on the outside of the LabTube from the microcontroller to the heater in revolver 3.

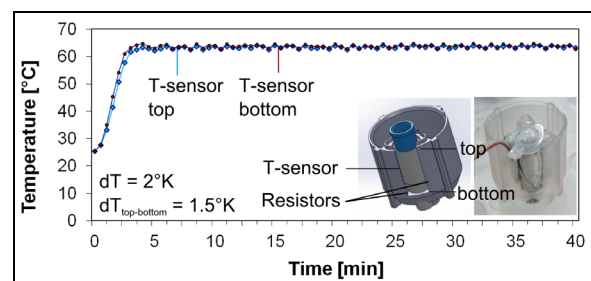


Figure 3. The heater consists of two SMD thick film resistors and an NTC resistor as a temperature sensor. The reaction chamber is surrounded by aluminium foil to ensure homogeneous temperature distribution. The temperature profile is measured at the top and bottom of the reaction chamber filled with  $150\mu\text{l}$  water. It is stable to  $\pm 2^\circ\text{C}$ .

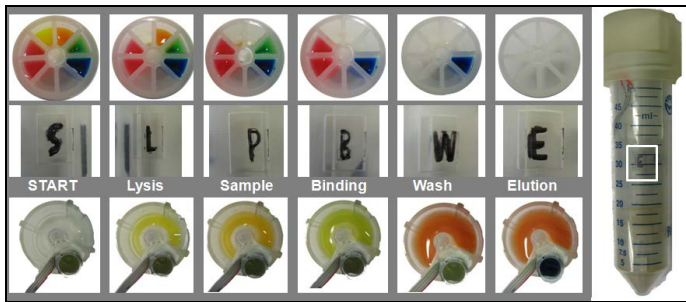


Figure 4. Mechanical and fluidic verification of the heated LabTube. Revolver 1 was filled with water colors instead of chemicals, in order to track their fluid flow. After running the system, the PCR tube had collected the desired amount of eluate (blue), whilst all the remaining liquids were transferred to the waste chamber (orange). The window (rectangle) indicates different processing steps.

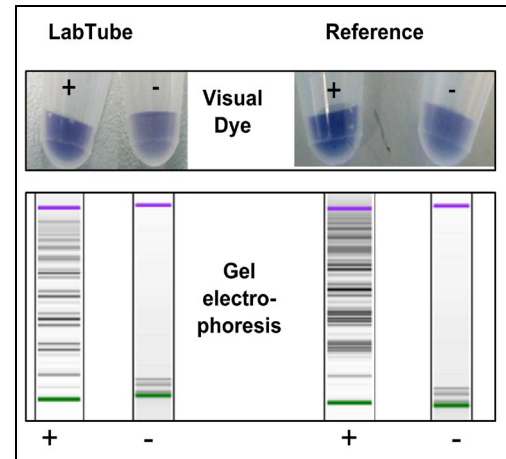


Figure 5. Results of a complete extraction and amplification of VTEC *E.coli* lysate in water ( $10^3$  copies) with the LabTube ( $n=3$ ). Both the LabTube and reference positive control show a color change and amplification product both visually and on the quantitative gel electrophoresis (Agilent 2100), whilst the negative control shows no color change or product.

## CONCLUSION

The DNA extraction and amplification system is disposable, fully closed and automated requiring only a single pipetting step. Since it runs on a standard laboratory centrifuge, the heated LabTube is suitable for analysis in low-resource areas, at production sites or sales locations. In the future, the heated LabSystem can be used for fully automated DNA extraction and amplification in a variety of food, environmental and consumer product analysis and medical diagnostics. The introduced heating system can further be easily parallelized and it enables the control of multiple independent heating zones within one LabTube. It is low-cost (<1\$) and is widely deployable, such as for other heating applications (e.g. pre-heating of lysis buffers), for electrochemical reactions and/or for quality control.

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