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Oral

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

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Introduction:
In this paper, we present a rapid, automated, low-cost and easy-to-use DNA-extraction and loop-mediated isothermal amplification method (LAMP) for verotoxin-producing (VTEC) *E.coli* that can be used in the field without specialized training in <1.5hrs. 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 optimize 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 extraction and amplification of *E.coli* (VTEC) was incorporated into the LabTube. 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 the diagnosis is often time-critical, rapid extraction methods and tests that can be used at the point-of-care (e.g. hospital or production site) are desirable.

Results:
As shown in Figure 1B, as little as 10^2 copies of VTEC DNA were extracted using the QIAamp Micro DNA kit, which yielded higher extraction efficiencies than other tested silica-column based kits. The extraction efficiency was 157±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, allowing a simpler and cheaper, disposable heating system to be used. The heating system was controlled and driven by a low-cost microcontroller (ATXMega) and a 3V CR-2 battery (Figure 2), which was able to yield 2W of power for more than 1hr. The system was able to withstand high centrifugation forces and showed full mechanical and fluidic functionality with the centrifugation protocol of the LabTube (Figure 3). The heater itself consisted of an SMD resistor and a NTC as heating and sensing elements. These elements were embedded in heat-conducting cement that surrounded an aluminum-wrapped sample chamber in order to achieve homogeneous temperatures. The achieved temperature profile was stable to ±2°C (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 within 40min from purple to blue upon reaction (Figure 5). The reaction product was confirmed with electrophoresis.

Outlook:
The introduced heating system can be easily parallelized and 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, for electrochemical reactions and/or for quality control. *Word Count: 499*

REFERENCES:

1. R. Zengerle; “Microfluidic “Apps” on Standard Lab-Instruments”; 16th International Conference on Miniaturized Systems for Chemistry and Life Sciences (µTAS), Okinawa, Japan, 28.10.–01.11.2012.
2. Waters, J. R., J. C. M. Sharp, and V. J. Dev., Infection caused by Escherichia coli O157:H7 in Alberta, Canada, and in Scotland: a five-year review, 1987-1991, 1994, Clin. Infect. Dis. 19:834-843.

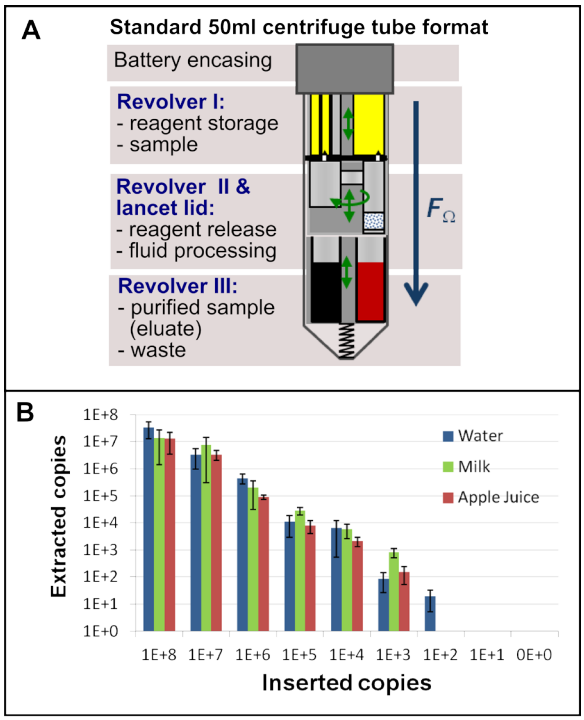

Figure 1: ***(A)*** *Schematic of the LabTube components.* ***(B)*** *DNA extraction of VTEC E.Coli (EDL 933) lysate in water, milk and apple juice inside the LabTube (n≥3). Using the QIAamp Micro DNA kit, the extraction limit is ≥10^2 in water and ≥10^3 copies in milk and apple juice as shown with qPCR.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

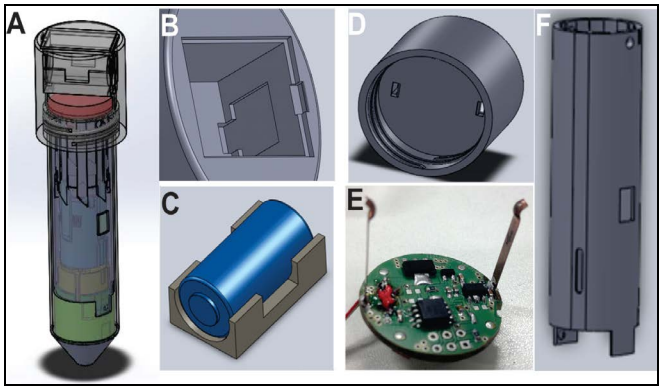

Figure 2: ***(A)*** *Complete LabTube with battery encasing.* ***(B)*** *Cap with holes for electrical contacts of the microcontroller to contact the battery sitting above it.* ***(C)*** *The battery is embedded in a soft constricted piece.* ***(D)*** *It is sitting in a cavity.* ***(E)*** *Below it 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 and via the hole to the heater in revolver 3.*

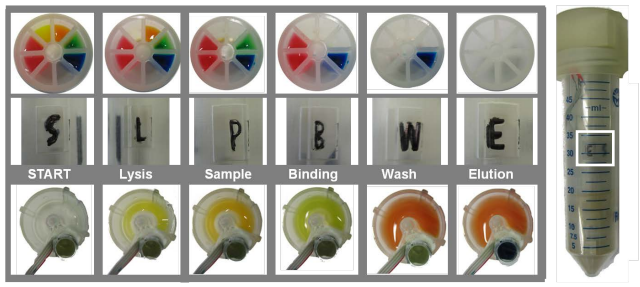

Figure 3: *Mechanical and fluidic verification of the modified, heated LabTube. Revolver 1 was filled with water colors instead of chemicals, in order to track their fluid flow. After running the system, the PCR tube had collected the desired amount of eluate (blue), whilst all the remaining liquids were transferred to the waste chamber (orange). The window (rectangle) indicates different processing steps.*

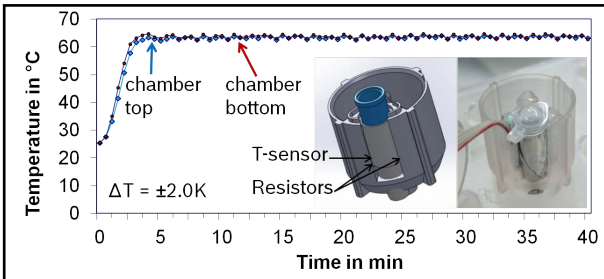

Figure 4: *The heater consisted of two SMD thick film resistors and an NTC resistor as a temperature sensor. The reaction chamber was surrounded by aluminium foil in order to ensure homogeneous temperature distribution. The temperature profile was measured at the top and bottom of the reaction chamber filled with 150µl of water. It is stable to ±2°C.*

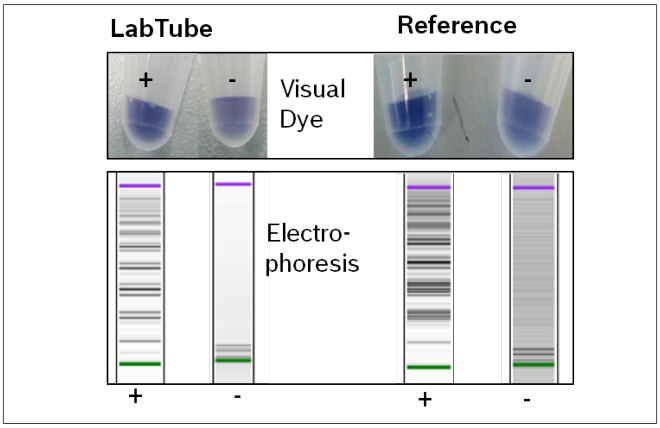

Figure 5: *Results of a complete extraction and amplification of VTEC E.coli lysate in water (10^3 copies) with the LabTube (n=3). Both the LabTube and reference positive control show a color change and amplification product both visually and on the quantitative gel electrophoresis (Agilent 2100), whilst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