Centrifugal LabTube platform for fully automated DNA purification and LAMP amplification based on an integrated, low-cost heating system

Melanie Hoehl[a,b,c,*], Michael Weißert[c,d], Arne Dannenberg[c], Thomas Nesch[e], Nils Paust[f], Felix von Stetten[f], Roland Zengerle[f], Alexander H. Slocum[a,b*,§] and Juergen Steigert[c,§]

[a] *Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, USA*
[b] *Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
[c] *Robert Bosch GmbH; Advanced Research and Engineering, Gerlingen, Germany*
[d] *University of Stuttgart, Microsystems Technology, Stuttgart, Germany*
[e] *Nesch Engineering UG (haftungsbeschränkt), Haigerloch, Germany*
[f] *HSG-IMIT, Freiburg, Germany*
* *Correspondence: melanie.hoehl@alum.mit.edu (phone 0049-711-81138232), slocum@mit.edu (phone 001-617-2530012)*
[§] *These authors contributed equally to this work.*

Abstract:

This paper introduces a disposable battery-driven heating system for loop-mediated isothermal DNA amplification (LAMP) inside a centrifugally-driven DNA purification platform (LabTube). We demonstrate LabTube-based fully automated DNA purification of as low as 100 cell-equivalents of verotoxin-producing *Escherichia coli* (VTEC) in water, milk and apple juice in a laboratory centrifuge, followed by integrated and automated LAMP amplification with a reduction of hands-on time from 45 to 1min. The heating system consists of two parallel SMD thick film resistors and a NTC as heating and temperature sensing elements. They are driven by a 3V battery and controlled by a microcontroller. The LAMP reagents are stored in the elution chamber and the amplification starts immediately after the eluate is purged into the chamber. The LabTube, including a microcontroller-based heating system, demonstrates contamination-free and automated sample-to-answer nucleic acid testing within a laboratory centrifuge. The heating system can be easily parallelized within one LabTube and it is deployable for a variety of heating and electrical applications.

Keywords:

Automated DNA extraction, DNA amplification, LAMP, heater, battery

1 Introduction

Contamination of water and food is a public health hazard that episodically causes thousands of deaths and each year sickens millions worldwide (Waters et al. 1994, Karmali et al. 2010). For example, verotoxin-producing *Escherichia coli* (VTEC) produce Shiga-like toxin, a main source of water and food borne illness (Karch et al. 2005). They are oftentimes found in contaminated water, meat, dairy products and juices (Beecher , Waters et al. 1994, Karch et al. 2005, Karmali et al. 2010). When infecting humans, they have been linked with the severe complication haemolytic uremic syndrome (Karch et al. 2005, Karmali et al. 2010). For example in 2012, 20 people got ill from drinking VTEC contaminated, unpasteurized milk in Oregon, USA (Beecher) and in 1996 66 people got sickened and one person died from VTEC contaminated apple-cider (European Commission of Health and Consumer Protection 2013). Further, the presence of any type of *E.coli* in foods or water is an indicator for fecal contamination and hence risk of exposure to other pathogens (Min and Baeumner 2002, Baeumner et al. 2003, Schuurman et al. 2007).

In order to ensure food and water safety and quality, processing and detection methods are desirable that can be used in the field to yield rapid time to results (Min and Baeumner 2002, Baeumner et al. 2003, Kost 2006, Lazcka et al. 2007, Lounsbury et al. 2013). Existing laboratory methods to detect many common relevant pathogens (such as immunoassays or qPCR) require specialized scientific equipment, a stable laboratory environment, a continuous refrigeration chain for reagents or antibodies, and/or specially trained staff to perform numerous manual steps, especially during the sample preparation procedure (Leonard et al. 2003, Lazcka et al. 2007, Schuurman et al. 2007, Nugen and Baeumner 2008, Velusamy et al. 2010, Park et al. 2011, Zuo et al. 2013). Automated sample preparation robots are commercially available, but they are expensive and often still require a specialized laboratory to perform manual pipetting steps for the downstream diagnostic reaction (Merel 2005, Lee et al. 2010, Phillips et al. 2012). They are therefore not practical for many users in outbreak locations, at the point-of-care or for low-throughput testing laboratories (Baeumner 2003, Lazcka et al. 2007, Park et al. 2011).

Any automated diagnostic test for small-scale use should rely on a simpler, more robust technology (Baeumner et al. 2003, Leonard et al. 2003, Kost 2006, Lazcka et al. 2007, Yager et al. 2008, Hoehl et al. 2012). There has thus been an effort to develop low-cost, automated diagnostic biosensors (e.g. nanotechnology-based sensors (Zhang et al. 2005) or microfluidic systems, such as droplet-based (Zhang et al. 2011, Chiou et al. 2013), centrifugal (Cho et al. 2007, Lutz et al. 2010), paper (Martinez et al. 2009, Fu et al. 2010, Zuo et al. 2013), capillary (Takayama et al. 1999, Yager et al. 2008, Gervais and Delamarche 2009), pneumatic (Legendre et al. 2006, Mohan et al. 2013) devices and lateral flow assays (Yager et al. 2008, Chen et al. 2010, You et al. 2011)). For the past decade, this has led to numerous publications about promising, early-stage technologies (Baeumner et al. 2003, Leonard et al. 2003, Kost 2006, Lazcka et al. 2007, Liu et al. 2010, Lucchi et al. 2010, Asiello and Baeumner 2011, Duarte et al. 2011, Karlsson et al. 2011, Park et al. 2011, Schudel et al. 2011, Matlock-Colangelo and Baeumner 2012, Lounsbury et al. 2013, Mohan et al. 2013). Despite some commercially available systems (e.g. Cepheid GeneXpert, Biocartis and. Abbott i-STAT), many of these systems still lack commercial maturity (Lazcka et al. 2007), especially when they include sample preparation steps (Wen et al. 2008). They further often require expensive hardware for optical readout or fluidic control and are usable for a small range of applications only (Yager et al. 2008).

The LabTube is a disposable cartridge for automated DNA purification in a laboratory centrifuge. The LabTube is based on disposable, polymeric modules integrated in a 50ml falcon tube (Kloke et al, 2014), in which the DNA purification workflow is automated by applying process-specific centrifugation-time-protocols to a centrifuge (Fig. 1). A centrifugally actuated ballpen mechanism induces a stepwise rotation of Revolver II with respect to the other cartridge components and a simultaneous up-down movement with respect to Revolver I. This way, thorns integrated on top of Revolver II sequentially release reagents from pre-storage cavities of Revolver I and the off-center placed outlet of Revolver II transfers the liquids to different zones of Revolver III for product-waste separation. The required centrifuge-time protocol is transferred to the centrifuge via a computer interface (here, RS 232). Unlike other commercially available DNA purification devices, the LabTube runs with a laboratory centrifuge, it is fully closed (hence minimizing cross-contamination risks) and it can be used to automate a variety of manual processes.

In this paper, we incorporated a disposable battery-driven heating system into the LabTube. A heating system is required for the pre-heating of lysis or elution buffers to increase the DNA purification efficiency inside the LabTube. Heating is also required for removing ethanol from the silica-matrix to prevent inhibition of downstream processes, such as PCR. It can also be used for downstream assays, such as DNA amplification or immunoassays.

As a first application example, fully automated DNA purification and subsequent isothermal loop-mediated DNA amplification (LAMP) of as low as 100 VTEC cell-equivalents in real samples (water, apple juice and milk) was integrated into the heated LabTube. The results are readout by a visual detection dye. This paper reports the first automated purification and amplification of bacteria and in food matrices inside the here-introduced, heated LabTube. To drive the DNA amplification reaction, a

battery-driven and microcontroller-based heating system was integrated. The heater was designed with common design principles and with finite element simulations. The introduced system is low-cost, disposable and fully closed, requiring a single pipetting step only, thereby reducing contamination risks. Unlike other fully automated systems, the heated LabTube only requires a single device, namely a laboratory centrifuge.

2 Materials and Methods

2.1 Battery Testing

Three different batteries were used for an endurance test: CR-2 (3V, Panasonic), 4LR44 (6V, Camelion) and A23 (12V, Camelion). The endurance test was performed at an initial power of 2.5W with a resistor combination consisting of SMD thick film resistors (3.6, 14.4 and 57.6Ω respectively). For the endurance test, both voltage, V, and current, I, over time, t, were recorded. By multiplication of the two electrical measures at every single point in time, the power, P, versus time, t, diagram shown in Fig. 2a could be derived.

2.2 Heater Unit Selection

The heater selection test consisted of heating 150µl of fluid up to 65°C using a constant power of about 2.5W (see ESI). The time to reach a final temperature was measured using a stopwatch and a temperature logger (Ebro, 40 TC02). As a heating unit a PTC resistor (EPCOS, 8.2Ω at 25°C) was tested using a constant voltage of 5V. Next, heaters that can be used in conjunction with a NTC thermistor as a temperature sensor were tested. Here, a controller module (Carel IR33), a 12V DC power supply and a motherboard with a temperature control circuit voltage regulator were set up in order to test different heating elements (see ESI).

2.3 Heat Transfer Simulations

Conductive heat transfer was modeled using the ANSYS Workbench 14.5 for finite element simulation. SolidWorks models of the heated LabTube were imported. Surface convection was set to 8 W/(m²*K) on all surfaces contacting the surrounding air. Radiation was neglected in the simulation, since the temperatures (~65°C) are low. Heat generation was set to 0.37 W and was modelled as internal volume heat generation evenly distributed between both heat dissipation resistors. The heat flow from the SMD resistors to its surroundings as well as the temperature range of the PCR mix was evaluated.

2.4 DNA Purification

Lysate of VTEC (EDL 933, Biotecon) was prepared by Biotecon GmbH and quantified by cell plating and counting. To prepare the samples, milk, water and apple juice were spiked with known amounts of VTEC lysate (EDL 933). Samples were extracted using the Micro DNA kit (Qiagen, Cat. no. 56304). In order to run the DNA purification in the LabTube, the silica column was inserted into Revolver II (Fig. 1). Revolver I was filled with the sample and Qiagen reagents: 100µl of sample, 125µl buffer ATL including 25µl Proteinase K, 300µl AL buffer (including 3ng/µl carrier RNA), 50µl ethanol, 20ul elution buffer and 450µl each of wash buffers AW1 and AW2. The LabTube for DNA extraction is made of stereolithographic parts in a 50ml centrifuge tube format. The DNA was extracted using a programmable laboratory centrifuge (Hermle, Z326K) with a swing-bucket rotor. For automated processing, a centrifugation-time protocol (altering centrifugal speeds between 40-6000g) is transmitted via a computer interface (RS232) to the centrifuge.

2.4.1 PCR

The extracted DNA was quantified using quantitative real-time PCR (Applied Biosystems, 7500). The following primers and probes(Karle et al. 2010) were used at 200nM in conjunction with the Quantifast master mix and Rox-dye (Qiagen, Cat.no. 204352):
Forward Primer - GGC AAT TGC GGC ATG TTC TTC C
Reverse Primer - TGT TGC ATT TGC AGA CGA GCC T
Probe:FAM-ATGCGAACGGCGGCAACGGCAACATGT -BHQ1
To start a reaction 18µl of master mix including 200nM of each primers and probes were mixed with 2µl of sample. The PCR was run with a 3min activation phase at 95°C, followed by 40 cycles with 10s denaturation and 40s annealing/elongation periods.

2.5 Isothermal DNA Amplification

LAMP amplification of VTEC was performed using a commercial kit (Mast Diagnostica, Cat.no. 67vtsck3). 130µl of master mix were placed into the reaction chamber in Revolver III at the beginning of the purification. The DNA was eluted in 20µl of liquid - hence the overall reaction volume was 150µl for LAMP amplification. The amplification occurred at 65°C for 40min after successful DNA purification. The heating was initiated by a microcontroller timer. Afterwards the color change of the visual detection dye (Mast Diagnostica) was inspected, with blue being a positive and purple being a negative result. The master mix contained 117µl reaction mix, 7µL visual detection dye and 7µL BST polymerase, which are all provided in the kit. A positive and negative (water only) control was always run in parallel in a thermomixer (Eppendorf, 5438).

2.5.1 Quantitative Electrophoresis

The amplification products of the reference reactions were quantified with electrophoresis on an Agilent 2100 bioanalyzer using the DNA1000 kit.

Process	Cell Lysis	DNA Elution	Ethanol Removal	Inactivation of Proteases	Isothermal LAMP Amplification
T (°C)	56, 70	70	78	90	65
Time (min)	15	10	3-5	5	30-60
Volume (µl)	170	20 – 100	Ca. 20-100	170	100-150

Table 1: Example processes requiring heating inside the LabTube

Battery	L (mm)	D (mm)	M (g)	V (V)	Q (mAh)
CR-2	15.6	27.0	10	3	750
4LR44	12.8	25.1	9	6	165
A 23	10.3	27.5	9	12	55

Table 2: Selected batteries

3 Electrical Design

3.1 Heating Requirements

The goal of this study was to develop a disposable LabTube heater to drive fully automated VTEC DNA amplification downstream of DNA purification from real sample matrices. The heater should be designed to have the potential for flexible use with other heating applications summarized in Table 1. These include pre-heating of lysis or elution buffers to increase DNA purification efficiencies inside the LabTube, removing ethanol from the silica-matrix to prevent inhibition of downstream processes (like PCR) and the inactivation of proteases. They also include heating for downstream assays, such as DNA amplification and immunoassays. Since for many of these applications, heating needs to be performed during DNA extraction, a heater for use inside the centrifuge - rather than in an external unit - is necessary. For broad usability of the heated LabTube, the heater should be made compatible with commercially available laboratory centrifuges, rather than with a custom centrifuge.

As a first example, VTEC DNA amplification was integrated into the LabTube. The LAMP reaction (Hara-Kudo et al. 2007) was chosen over other amplification methods, such as PCR (Hill et al. 2008). This is because the desired test result does not have to be quantitative (a simple yes or no answer above a certain threshold suffices (Europäische Kommission 2004, Gill and Ghaemi 2008)), yet it has to be sensitive, temperature robust and specific for the target organism. The LAMP reaction was chosen since unlike PCR, it does not require thermal cycling and it is more temperature robust than other isothermal amplification methods (Francois et al. 2011). It was shown to be stable at 67±5°C, see Fig. S1. Additionally, reduced time to result of LAMP compared with PCR allows timely and goal directed decision making.

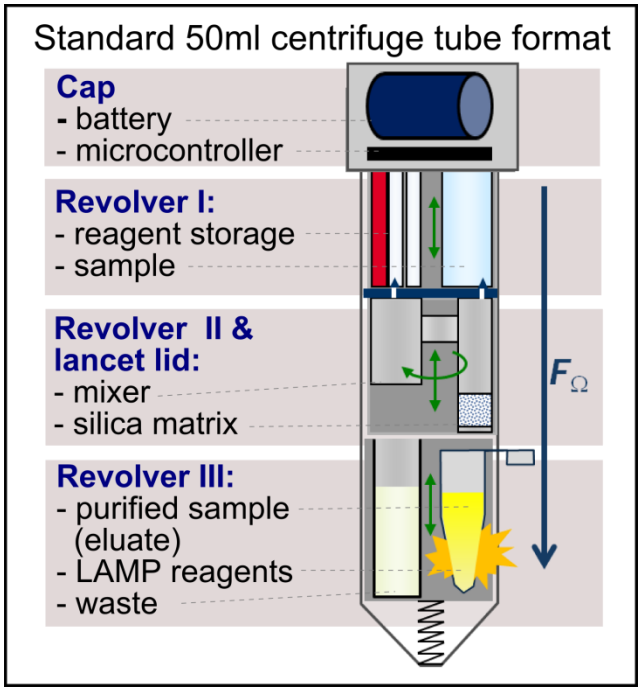

Fig. 1: Heated LabTube showing three different Revolvers and a cap. The DNA amplification takes place in the sample chamber of Revolver III (yellow)

For LAMP amplification inside the LabTube, the heater needs to heat 50-150µl liquid up to 65°C essentially as fast as possible and it needs to be able to keep the temperature for at least 40 minutes. The heating process takes place in Revolver III of the LabTube, where the extracted DNA is collected. Additionally, the entire heating system needs to be temperature stable (±2°K), have a ramping time <10min, be disposable, cost-efficient (<$1 in mass production) and bioassay-compatible. It also needs to be small enough to fit into the LabTube, which has the format of a 50ml centrifuge tube and a cap with a maximum height of 18.5mm and an outer diameter of 70mm (such as to still fit into the centrifuge).

3.2 Power Supply

Generally, heat can be generated electrically via resistive or inductive methods, chemically (Weigl et al. 2008a, Weigl et al. 2008b, Liu et al. 2011) (e.g. using exothermic reactions) and/or using rotational friction forces inside the centrifuge. The developed power supply should be compatible with commercial laboratory centrifuges without requiring retrofits, in order to ensure the system's broad applicability. To get the best compromise between economic, size and performance features, a battery-driven heating system was chosen over other methods. Initially, suitable batteries for isothermal LAMP DNA amplification were selected based on the above requirements for temperature stability, ramping times, price and physical size:

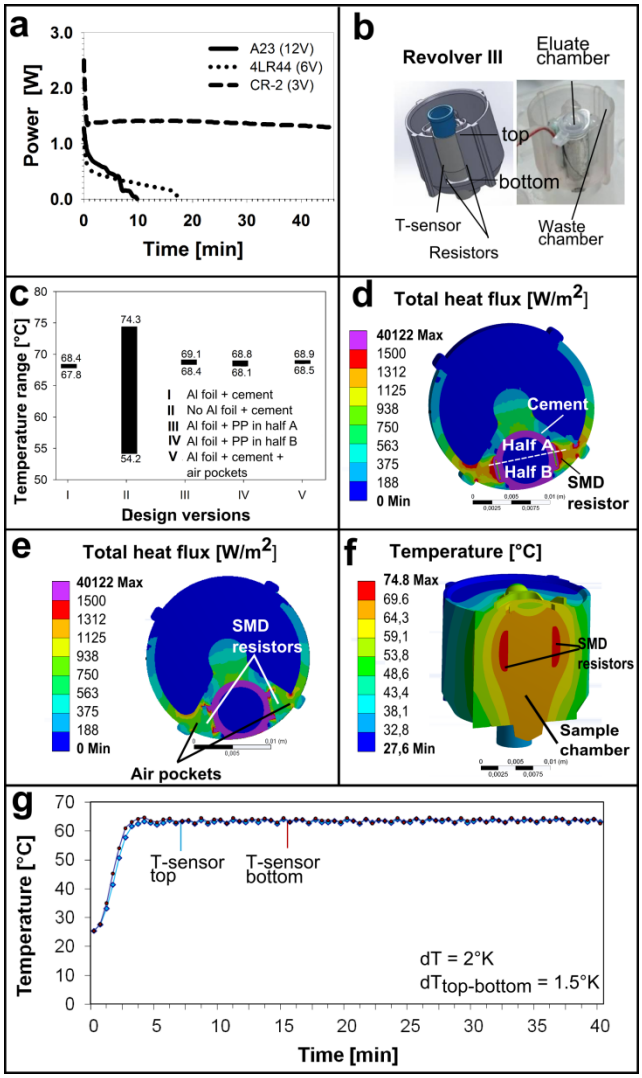

Fig. 2: a) Battery stability tests with a constant resistance and initial power output of 2.5W. Power, the product of the measured voltage and current, is plotted over time. The CR-2 battery yields a constant power output (~ 1.5W), whilst A23 and 4LR44 collapse after <10min. **b)** The heater of Revolver III consists of two SMD thick film resistors and an NTC thermistor as a temperature sensor. The PCR tube contains the sample. **c)** Simulated temperature range for different heater insulation designs. **d)** Total heat flux for design I (aluminium foil and heat conducting cement) and **e)** for air pockets next to the SMD resistors (design V). **f)** The simulated temperature distribution for the used design V. **g)** Experimentally measured temperature range inside the PCR tube. The temperature profile is measured at the top and bottom of the PCR tube filled with 150µl of water. It is stable to 65±2°C

It was experimentally determined that heating up a 150µl sample of water to 65°C within 2 minutes using standard resistors as heating elements, required a constant power input of ~2.5W and ~1.5W to keep the temperature stable for 40 min (no additional insulation). According to this result, the current drawn from a battery with a nominal voltage between 1.5V-12V during the initial heating period is between 670-80mA, respectively. Required electric charges are between 80mAh for 12V batteries and 580mAh for 3V batteries. 53 batteries were screened based on the above requirements (Ralf Hottmeyer Gmbh 2012).

Based on the evaluation, only two batteries were able to fulfil the requirements (Table 2): CR-2 (3V) and 4LR44 (6V). These batteries are available as rechargeables, which ensures that the disposable LabTube remains environmentally friendly. The A23 (12V) battery contains a charge of 55mAh, which is only slightly lower than the calculated requirements and it was also chosen, as good thermal insulation could reduce the required charge. An endurance test for the three selected batteries was conducted at an initial power of ~2.5W (at the very beginning) and the voltage and current were recorded over time (Fig. S2). The test was performed for 40 minutes to ensure that the corresponding battery can provide sufficient power to drive the LAMP reaction. As the primary interest was to evaluate the battery performance over time, the power released by the battery is shown in Fig. 2a. As indicated, only the CR-2 battery sustained the test, whilst the current for the other two decreased dramatically after less than 10min. It was also shown that the CR-2 battery cannot sustain the initial power of 2.5W, but instead yields a constant power of ~1.5W. This implies that good thermal insulation is required to ensure proper heating.

3.3 Heater and Sensor

Different heaters and sensors were experimentally compared (at ~2.5W). Whilst the heater candidates, a PTC resistor and Nickel wire, could not achieve the required temperature profile (65°C), both a SMD thick film resistor, as well as a heating foil were able to heat the liquid up to 65°C within ~1.5min and keep the temperature within a tolerance of ±0.5°C (see ESI section 3). Due to size and cost considerations, the SMD thick film resistor was chosen. Using the SMD resistor, a heating system was set up in Revolver III of the LabTube, where isothermal DNA amplification should take place inside a PCR tube. The heating units were placed outside the PCR tube with no contact to the DNA amplification reagents, in order to avoid their inhibition by the release of interfering substances from the heater parts. The heater consists of two SMD thick film resistors (5.6Ω, Yageo), which are connected in parallel. In the centre between the two resistors, the NTC resistor is located to modify the voltage over the 1.2kΩ serial resistor, depending on its temperature values (see Fig. 2b and ESI, 3). To reduce the power requirements of the battery to or below ~1.5W, thermal insulation was required.

To optimize the insulation design, the steady-state, conductive, thermal response of the system for different

packaging options was simulated in ANSYS. Apart from optimizing the heater insulation packaging, the ANSYS model is a useful tool to efficiently design heaters for other applications inside the LabTube.

It was observed by modeling that in the presence of liquid in the waste chamber of Revolver III, convective heat losses become significant. To avoid convective heat losses, a 3mm air gap was added between the waste and eluate chamber both experimentally and in the simulation. To verify the ANSYS model, simulated temperatures were compared with experimental data of different liquid volumes at various target temperatures. Fig. 2c depicts the effect of design alterations on the temperature profile inside the reaction chamber. Wrapping the PCR-tube in aluminium foil (I) instead of PP (II) reduces the temperature variation from 20.1 to 0.6°K. To embed the heating units, different materials (PP and heat conducting cement) were compared by simulation. In designs I and II, the heater parts are embedded in heat conducting cement. Replacing inner half A (design III) or outer half B (design IV) of the heat conducting cement with PP, has a small effect only: it increases the temperature variation from 0.6 to 0.7°K (Fig. 2c; the halfs are defined in Fig. 2d). Based on these results, the heat flux in the reaction chamber for design I (aluminium foil and heat conducting cement) was simulated, as shown in Fig. 2d. As indicated in Fig. 2d, maximum heat losses occur next to the SMD resistors. To reduce the heat flux, the effect of adding air pockets (Fig. S7) on the heat flux was simulated. Fig. 2e indicates that air pockets reduce the heat losses next to the SMD resistors. The temperature variation is slightly reduced to 0.4°K (from 0.6°K) and the mean temperature is increased by 0.6°K (Fig. 2c, V). The optimized design V (aluminium foil, heat conducting cement and air pockets) was therefore selected. Fig. 2f shows the simulated, achieved temperature profile is inside Revolver III during LAMP amplification using 0.37W of power for 150µl of water.

Next, the simulation results for design V were confirmed experimentally. As depicted in Fig. 2g, a stable temperature profile of 65±2°C is reached after ~3 minutes, requiring ~0.3W. The time needed for the heating process is doubled from the initial heater selection tests (see above), mainly due to the presence of thermally conductive cement and the aluminium cover, which themselves need to be heated up first. The heat can be held at the target temperature for at least 40 minutes. To test the biocompatibility of the system, 150µl of VTEC LAMP master mix, target DNA and visual detection dye (Mast Diagnostica) were heated up to 65°C and kept at this temperature for 40 minutes (Fig. 2g). For the positive control, a color change of the visual dye was detected and the desired product showed up on electrophoresis; whilst nothing was detected in the negative control. It was therefore concluded that the heating system is bioassay-compatible. Additionally, isothermal DNA amplification was successfully repeated with different

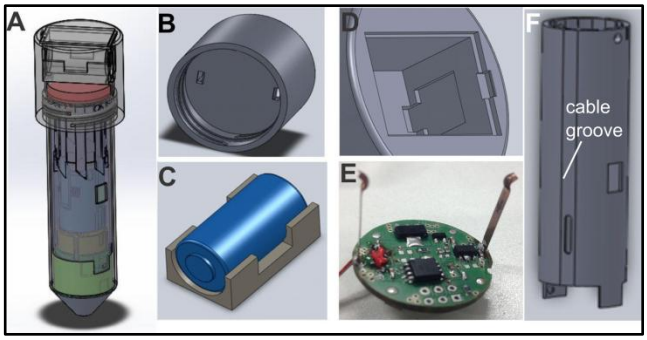

Fig. 3: a) Complete LabTube with battery encasing. **b)** Cap with holes for electrical contacts of the microcontroller to contact the battery sitting above it. **c)** The battery is embedded in a soft constricted piece. **d)** It is sitting in a cavity. **e)** Below it sits the round circuit board with the microcontroller. **f)** LabTube encasing with a hole for cables. The cable runs in a groove along the LabTube from the microcontroller and via the hole to the heater in Revolver 3

volumes (50µl and 100µl) of reagent, demonstrating that the heating system can also be run at lower volumes with shorter heating times (~1.5 and 2min, respectively), yielding temperatures stable at 65±2°C.

3.4 Heater Control

In order to keep the system flexible, the heating system is controlled with a microcontroller rather than with a discrete analogue circuit. This allows the user to change control parameters and/or applications flexibly and to perform several heating applications independently and in parallel. Because a CR-2 battery with 3V supply voltage is used, a microcontroller with an operating range beginning at 1.6V was chosen. To keep the costs low, a microcontroller with the minimal pin number is employed, having a retail sales price less than $0.5 and correspondingly lower in mass production (ATXmega, ATMEL). A round circuit board with the dimensions of the screw cap of a 50ml BD tube was designed using the program Eagle (Fig. 3e). The microcontroller was programmed using C++ and it can be set to different temperature profiles, as well as ramping times and start/stop times. Details of the microcontroller program may be found in the supplementary online material (ESI, section 4).

4 Mechanical Design

Encasings for the CR-2 battery, as well as for the circuit board were designed using Solid Works (Fig. 3). The encasing has a geometry of a 50ml falcon tube with a screw cap that holds the battery. It has a height of 18.5mm, which is the maximum height that still fits into the centrifuge. The encasing was rapid- prototyped using stereolithography and it consists of walls <3mm, allowing it to sustain high centrifugal acceleration of up to 6,000g. The battery sitting

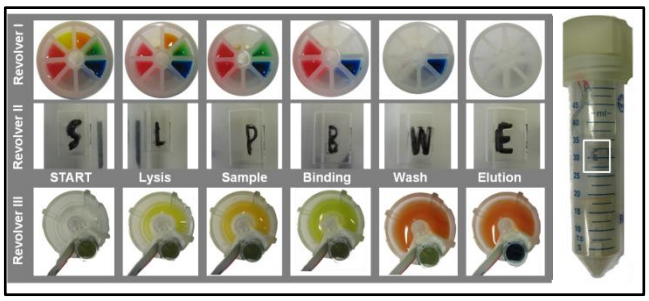

Fig. 4: Mechanical and fluidic verification of the modified, heated LabTube. Revolver 1 is filled with water colors instead of chemicals, in order to track their fluid flow. After running the system, the PCR tube has collected the desired amount of eluate (blue), whilst all the remaining liquids are transferred to the waste chamber (orange). The window (rectangle) indicates different processing steps. The battery encasing and cables therefore do not inihibit the mechanical functionalities

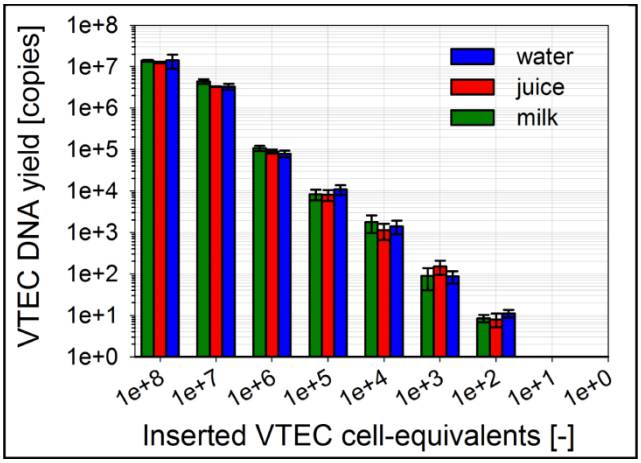

Fig. 5: DNA purification in the LabTube for VTEC in water, milk and apple juice

in the cap cavity (Fig. 3a and b) is embedded in a soft constricted piece located in the bottom of the cap (Fig. 3c) with the electrical contacts fitting through two holes into the battery cavity above (Fig. 3b and d). The heating system in Revolver III is connected with the microcontroller (Fig. 3e) and battery through a 4-core flat ribbon cable. Because the Revolvers move up and down in parallel to the centrifugal force during operation, the cable is arranged such as to not obstruct the pen-mechanism of the remaining Revolvers. Hence, the cable runs in a groove along the outside of the LabTube and it is connected with the heater through a hole at the bottom (Fig. 3f). To reduce waste and to ensure environmental friendliness, it is suggested to reuse the battery cap (consisting of a rechargeable battery and the microcontroller) between LabTube runs, whilst the LabTube including the heater and cables could be disposed of after each run.

5 Results

5.1 Mechanic, Fluidic and Electric Verification During Operation

Mechanic, fluidic and electric functionalities of the here-introduced modified, heated LabTube (including the cap and cables) were verified by running the system inside the centrifuge. Here, pen mechanics, actuated by centrifugal forces, stepwise rotate Revolver II by changing the centrifugal acceleration, which affords opening and closing of fluidic paths through the stack (Kloke et al, 2014). The mechanics of the modified LabTube system was successfully verified as Revolver II moved from the start (S) to end (E) position during the centrifugation protocol, whilst all components remained intact (Fig. 4).

The fluidics of the system was also verified. Revolver I was filled with water colors instead of chemicals in order to track the 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) (Fig. 4).

During this run, the electric heating system also performed as desired: at the end of the run the blue eluate sample (150µl) achieved a temperature of 64±2°C in the top and bottom of the reaction chamber.

It was concluded that the here-introduced, heated LabTube design is functional both mechanically, fluidically and electrically inside the centrifuge.

5.2 DNA Purification

DNA purification was performed with the heated LabTube by extracting log-dilutions of 10^8-0 lysed VTEC cells in water, milk and apple juice. A manual reference was always run in parallel as a control. The eluates were subsequently quantified using qPCR. As shown in Fig. 5, the purification limit is 10^2 inserted cell-equivalents for water and 10^3 inserted cell-equivalents for apple juice and milk. The tested efficiency of VTEC DNA purification is 147±37% compared to the manual reference (which is normalized to 100±18%). This result is expected to be caused by a better mixing efficiency of the binding buffer with the lysate using the density relocation mixer of the LabTube, compared with manual vortexing. In this study, cell-lysates were used due to high infectious risk of VTEC. Figure S13 shows that for whole, non-toxic *E.coli* cells (41447), the extraction limit inside the LabTube is 10^2 cells in water and 10^3 cells in milk, with an efficiency of 91±17%. The lower efficiency for whole cells is likely caused by the less efficient lysis step compared with the manual control. These results represent the first extraction of bacteria and in food matrices using the LabTube. In the future, the achieved detection limit could be reduced by inserting 4ml instead of 100µl of sample. If lower detection limits are required, pre-enrichment or centrifugal filtration

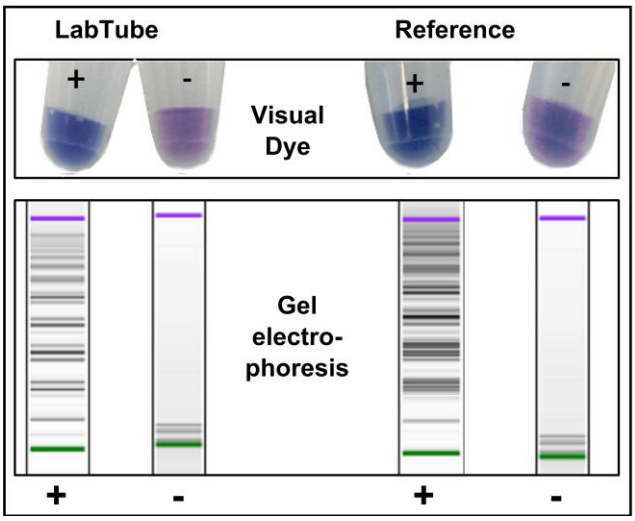

Fig. 6: Results of the combined purification and amplification of VTEC in the LabTube. Both the LabTube and reference positive control show a visual color change and amplification product via electrophoresis, whilst the negative control shows no color change or product

(e.g. using Viva Spin, Sartorius filters) should be employed.

5.3 DNA Amplification

Complete DNA purification protocols were run in the LabTube by inserting 10^3 cell-equivalents of lysed VTEC, extracting its DNA and amplifying the purified DNA by LAMP (n=3). Positive and negative controls were run in parallel in a thermomixer at 65°C for 40min. The amplified DNA was detected with a visual dye. The results were verified using quantitative electrophoresis. As can be seen in Fig. 6, the negative control stayed purple, whilst the positive control changed its color to dark blue for all three runs. Moreover, quantitative electrophoresis identified the desired amplification product in the positive sample, whilst the negative contained no product. The overall time to result for DNA purification and amplification was 85min. Compared with the manual reference, the hands-on time was reduced from 45 to 1min involving only a single instead of thirteen pipetting steps, as well as only a single instead of five loading/unloading steps of the centrifuge.

5.4 Summary

In summary, a disposable, microcontroller-based, battery-driven heating system was introduced for use inside the LabTube, a microfluidic DNA purification platform that runs with a laboratory centrifuge. The heating system is driven by a 3V battery and controlled by a microcontroller. It consists of two parallel SMD thick film resistors and a NTC thermistor as heating units and temperature sensors. The heater was designed with common design principles and with finite-element simulations. The model is a useful tool to efficiently design heaters for other applications inside the LabTube. As a first application, the heater was used for isothermal DNA amplification (LAMP) of lysed VTEC cells following their automated DNA purification inside the LabTube. In this fully closed and automated system, the LAMP reagents are purged into the elution chamber and the amplification reaction starts immediately after the DNA eluate is collected inside this chamber. For the first time, as little as 100 VTEC cell-equivalents were automatically LabTube-purified, LAMP amplified and readout using a visual detection dye.

6 Discussion

In this study, the heated LabTube was introduced for fully-automated DNA purification and LAMP amplification of VTEC in food matrices. The heated LabTube is fully closed, hence minimizing contamination risks. Reduced time to result of the LAMP amplification compared with PCR, allows timely and goal directed decision making. Since the heating system is low-cost (<1$ in mass production), it can be used as a disposable unit with costs comparable with manual methods. Alternatively, costs can be further reduced and environmental friendliness can be increased, if the battery cap (consisting of a rechargeable battery and the microcontroller) is reused. The heated LabTube is a fully automated sample-to-answer system and reduces manual labor time from 45 to 1min, requiring a single pipetting and centrifuge-loading step only. Instead of requiring expensive devices for DNA purification and amplification, the heated LabTube only requires a laboratory centrifuge and it is easily scalable (up to 20 LabTubes/centrifuge run). The introduced heating system further is broadly deployable, e.g. to improve DNA extraction efficiencies (by heating lysis or elution buffers) and purities (by removing ethanol from the silica matrix) for difficult to extract matrices.

The heated LabTube can be improved and extended in a variety of ways. A reference color pad could be attached to the outside of the LabTube in order to clearly differentiate positive from negative results (as well as gradients thereof). The LAMP reagents in the amplification chamber could be pre-stored in lyophilized form for long-term storage. To perform different amplification reactions in parallel, e.g. for multiplexing, multiple reagent chambers could be integrated into Revolver III (e.g. a coiled-up PCR 8-cap strip containing different primer sets for multiplexing).

If a rechargeable battery is used, the user could be equipped with a device indicating the charging status of the battery before use. The here introduced, heated LabTube has the advantage that it is operated with a commercially available laboratory centrifuge, hence affording broad deployment even in resource-limited environments. For less cost-driven scenarios, a pricier, custom processing device could be developed, in which the here developed heater design is powered via sliding ring contacts, by electromagnetic induction or with microwaves.

Independently, the developed method for LAMP amplification inside the LabTube is broadly applicable to many targets and it could be employed in a variety of food, medical and environmental diagnostic applications. The heating system affords automation of a variety of processes requiring temperature control inside the LabTube. In the future, other types of nucleic acid amplification reactions (e.g. PCR, RPA, etc.), immunoassays (e.g. ELISA) or even chemical synthesis reactions could be integrated. The heating system could also be used to remove ethanol from the eluate or the column; or for the pre-heating of lysis buffers to increase the quality and yield of difficult to extract matrices. Since the heater can be easily parallelized, multiple independent heating zones could be integrated and controlled in a single LabTube. The introduced ANSYS model could serve as a useful tool to efficiently design these heaters. The microcontroller and battery-based system could further be used to control electrochemical reactions, to perform electrical measurements (e.g. pH) or even for quality control.

7 Conclusion

Overall, the introduced heated LabTube for DNA purification and LAMP amplification is fully automated and integrated. Unlike many analytic strategies, the system offers application flexibility, yet is low-cost and employs laboratory equipment. The system could help hasten more testing and analysis at the location of an outbreak, the production site or at the point-of-care, which could increase safety, reduce contamination outbreaks, as well as the waste of precious resources. The heated LabTube could be used to increase safety and product quality in many applications, such as food applications, environmental and product safety and in medical diagnostics.

Notes and References

Acknowledgements:

We thank the Harvard-MIT Division of Health Sciences and Technology, as well as the Legatum Center at MIT for support. Thanks to the CR/ARY2 team at Robert Bosch GmbH, particularly F. Laermer, B. Faltin, J. Hoffmann and K. Lemuth. We thank the staff at Nesch Engineering for the productive collaboration. We also thank H.-E.Manneck from Mast Diagnostica for technical support.

Abbreviations:

E. coli (Escherichia coli); LAMP (loop-mediated isothermal DNA amplification); VTEC (verotoxin producing E. coli); DNA yield (extracted DNA copies, which here are quantified by qPCR)

Electronic Supplementary Information:

The ESI contains details on the LAMP stability testing, the power supply, heater selection and control, as well as LabTube extraction of whole *E.coli* cells.

References:

P. J. Asiello, A. J. Baeumner, Lab Chip **11**, (2011)
A. J. Baeumner, An. Bioan. Chem. **377**, (2003)
A. J. Baeumner, R. N. Cohen, V. Miksic, J. Min, Biosens. Bioelectron. **18**, (2003)
C. Beecher, 19 Ill with E. Coli in Oregon Raw Milk Outbreak. (Food Safety News, www.foodsafetynews.com/ 2012/04/post-5/ Accessed May 4th 2013
D. Chen, M. Mauk, X. Qiu, C. Liu, J. Kim, S. Ramprasad et al., Biomed. Microdev. **12**, (2010)
C.-H. Chiou, D. Jin Shin, Y. Zhang, T.-H. Wang, Biosens. Bioelectr. (2013)
Y.-K. Cho, J.-G. Lee, J.-M. Park, B.-S. Lee, Y. Lee, C. Ko, Lab Chip **7**, (2007)
G. R. Duarte, C. W. Price, B. H. Augustine, E. Carrilho, J. P. Landers, An. Chem. **83**, (2011)
Europäische Kommission, Verordnung der europäischen Kommission über mikrobiologische Kriterien für Lebensmittel, Milchverordnung (EU, Brussels, 2004), 6(3.3)
European Commission of Health and Consumer Protection, Opinion of the scientific committee on veterinary measures relating to public health on verotoxigenic E. coli (VTEC) in food stuffs (ECHCP, 2013), 1
P. Francois, M. Tangomo, J. Hibbs, E. J. Bonetti, C. C. Boehme, T. Notomi, M. D. Perkins, J. Schrenzel, FEMS Immunol. Med. Microbiol. **62**, (2011)
E. Fu, B. Lutz, P. Kauffman, P. Yager, Lab Chip **10**, (2010)
L. Gervais, E. Delamarche, Lab Chip **9**, (2009)
P. Gill, A. Ghaemi, Nucleos Nucleot Nucl **27**, (2008)
Y. Hara-Kudo, J. Nemoto, K. Ohtsuka, Y. Segawa, K. Takatori, T. Kojima, M. Ikedo, J. Med. Microbiol. **56**, (2007)
J. Hill, S. Beriwal, I. Chandra, V. K. Paul, A. Kapil, T. Singh et al., J. Clin. Microbiol. **46**, (2008)
M. M. Hoehl, P. J. Lu, P. A. Sims, A. H. Slocum, J. Agric. Food. Chem. **60**, (2012)
H. Karch, P. I. Tarr, M. Bielaszewska, Int. J. Med. Microbiol. **295**, (2005)
M. Karle, J. Miwa, G. Czilwik, V. Auwärter, G. Roth, R. Zengerle, F. Von Stetten, Lab Chip **10**, (2010)
J. M. Karlsson, T. Haraldsson, S. Laakso, A. Virtanen, M. Maki, G. Ronan, W. Van Der Wijngaart (2011) PCR on a PDMS-based microchip with integrated bubble removal. In Solid-State Sensors, Actuators and

Microsystems Conference (TRANSDUCERS), 2011 16th International. (ed.), Vol. pp. 2215-8, IEEE,
M. A. Karmali, V. Gannon, J. M. Sargeant, Vet. Microbiol. **140**, (2010)
A. Kloke, A. R. Fiebach, L. Drechsel, S. Zhang, S. Niekrawietz, M. Hoehl, R. Kneusel, K. Panthel, J. Steigert, F. von Stetten, R. Zengerle, N. Paust, Lab Chip, doi: 10.1039/C3LC51261D (2014).
G. J. Kost, Point Care **5**, (2006)
O. Lazcka, F. Campo, F. X. Munoz, Biosens. Bioelectr. **22**, (2007)
J.-H. Lee, Y. Park, J. R. Choi, E. K. Lee, H.-S. Kim, Yonsei Med. J. **51**, (2010)
L. A. Legendre, J. M. Bienvenue, M. G. Roper, J. P. Ferrance, J. P. Landers, An. Chem. **78**, (2006)
P. Leonard, S. Hearty, J. Brennan, L. Dunne, J. Quinn, T. Chakraborty, R. O'kennedy, Enzyme Microb. Technol. **32**, (2003)
C. Liu, M. G. Mauk, R. Hart, X. Qiu, H. H. Bau, Lab Chip **11**, (2011)
K. J. Liu, M. V. Brock, I.-M. Shih, T.-H. Wang, J. Am. Chem. Soc. **132**, (2010)
J. A. Lounsbury, A. Karlsson, D. C. Miranian, S. M. Cronk, D. A. Nelson, J. Li et al., Lab Chip **13**, (2013)
N. W. Lucchi, A. Demas, J. Narayanan, D. Sumari, A. Kabanywanyi, S. P. Kachur, J. W. Barnwell, V. Udhayakumar, PLoS One **5**, (2010)
S. Lutz, P. Weber, M. Focke, B. Faltin, J. Hoffmann, C. Müller et al., Lab Chip **10**, (2010)
A. W. Martinez, S. T. Phillips, G. M. Whitesides, E. Carrilho, Anal. Chem. **82**, (2009)
L. Matlock-Colangelo, A. J. Baeumner, Lab Chip **12**, (2012)
P. Merel, J. Assoc. Lab. Autom. **10**, (2005)
J. Min, A. J. Baeumner, Anal. Biochem. **303**, (2002)
R. Mohan, A. Mukherjee, S. E. Sevgen, C. Sanpitakseree, J. Lee, C. M. Schroeder, P. J. Kenis, Biosens. Bioelectr. (2013)
S. R. Nugen, A. Baeumner, Anal. Bioanal. Chem. **391**, (2008)
S. Park, Y. Zhang, S. Lin, T.-H. Wang, S. Yang, Biotech. Adv. **29**, (2011)
K. Phillips, N. Mccallum, L. Welch, Forensic Sci. Int. Genet. **6**, (2012)
Ralf Hottmeyer Gmbh, Vergleichslisten und Informationen für Knopfzellen und Batterien Würselen, 2012),
B. R. Schudel, M. Tanyeri, A. Mukherjee, C. M. Schroeder, P. J. Kenis, Lab Chip **11**, (2011)
T. Schuurman, A. Roovers, W. K. Van Der Zwaluw, A. A. Van Zwet, L. J. Sabbe, A. M. D. Kooistra-Smid, Y. T. Van Duynhoven, J. Microb. Meth. **70**, (2007)
S. Takayama, J. C. Mcdonald, E. Ostuni, M. N. Liang, P. J. Kenis, R. F. Ismagilov, G. M. Whitesides, Proc. Natl. Acad. Sci. U.S.A. **96**, (1999)
V. Velusamy, K. Arshak, O. Korostynska, K. Oliwa, C. Adley, Biotechnol. Adv. **28**, (2010)
J. R. Waters, J. C. Sharp, V. J. Dev, Clin. Inf. Dis. **19**, (1994)
B. Weigl, G. Domingo, P. Labarre, J. Gerlach, Lab Chip **8**, (2008a)
B. H. Weigl, G. Domingo, J. Gerlach, D. Tang, D. Harvey, N. Talwar et al., MOEMS-MEMS 2008 (2008b)
J. Wen, L. A. Legendre, J. M. Bienvenue, J. P. Landers, An. Chem. **80**, (2008)
P. Yager, G. J. Domingo, J. Gerdes, Annu. Rev. Biomed. Eng. **10**, (2008)
D. J. You, K. J. Geshell, J.-Y. Yoon, Biosens. Bioelectron. **28**, (2011)
C.-Y. Zhang, H.-C. Yeh, M. T. Kuroki, T.-H. Wang, Nature Mat. **4**, (2005)
Y. Zhang, S. Park, K. Liu, J. Tsuan, S. Yang, T.-H. Wang, Lab Chip **11**, (2011)
P. Zuo, X. Li, D. C. Dominguez, B.-C. Ye, Lab Chip **13**, (2013)