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FOR FOOD SAFETY BASED ON AUTOMATED DNA
EXTRACTION, AMPLIFICATION AND READOUT*

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LOW-COST BACTERIAL DETECTION SYSTEM FOR FOOD SAFETY BASED ON AUTOMATED DNA EXTRACTION, AMPLIFICATION AND READOUT

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ABSTRACT

To ensure food, medical and environmental safety and quality, rapid, low-cost and easy-to-use detection methods are desirable. Here, the LabSystem is introduced for integrated, automated DNA purification and amplification. It consists of a disposable, centrifugally-driven DNA purification platform (LabTube) and the subsequent amplification in a low-cost UV/vis-reader (LabReader). In this paper, food safety was chosen as the first sample application with pathogenic verotoxin-producing (VTEC) *Escherichia coli* (*E.coli*) in water and milk, and the product-spoiler *Alicyclobacillus acidoterrestris* (*A. acidoterrestris*) in apple juice as sample organisms. DNA was amplified qualitatively using isothermal loop-mediated DNA amplification (LAMP) and quantitatively using real-time PCR. By optimizing manual purification protocols inside the LabTube, as little as 45 inserted DNA copies were extracted from *E.coli* and *A.acidoterrestris* lysates in real samples (milk, juice and water). To run isothermal DNA amplification (LAMP) and PCR inside the LabReader, temperature control as well as data analysis methods were implemented. Combined detection limits for DNA purification and amplification from bacteria lysates in real samples at 10^2 - 10^3 inserted copies were achieved. The demonstrated LabSystem runs with standard laboratory equipment and reduces hands-on times from 30min to 3min.

KEYWORDS: Automated DNA extraction, DNA purification, DNA amplification, optical readout, PCR, LAMP

INTRODUCTION

Contamination of foods is a public health hazard that episodically causes thousands of deaths and sickens millions worldwide [1]. E.g., verotoxin-producing (VTEC) *E.coli* are a major source of foodborne illness. Product spoilers, like *Alicyclobacillus*, do not cause illness but great monetary losses to the juice industry [2]. To ensure food safety and quality, rapid, low-cost and easy-to-use detection methods are desirable. Here, the LabSystem is introduced for integrated, automated DNA purification and amplification. Recently, the basic mechanic principle of the LabTube, a disposable DNA purification platform that runs in a laboratory centrifuge, was introduced [3]. Here, DNA purification for *E.coli* and *Alicyclobacillus* lysates from real samples (milk, juice and water) as little as 45 copies was established for the first time inside the LabTube. In the LabSystem, the purified DNA was transferred into the LabReader, which consists of a low-cost, LED-based UV/Vis scheme that allows for simultaneous readout of four wavelengths [4]. The LabReader was modified to perform and automatically analyze loop-mediated isothermal DNA amplification (LAMP) and PCR.

EXPERIMENTAL

DNA purification: DNA was extracted from water, apple juice and milk samples with known amounts of cell lysate. *E.coli* (O157:H7) and *A. acidoterrestris* lysate was purchased from Bioteccon Diagnostics GmbH at known concentrations. All processing steps were performed at room temperature and in a Hermle swing-bucket centrifuge (Z326-K) using the QiaAMP DNA Micro kit. A manual reference in a swing bucket rotor was always run in parallel as a control. After DNA purification, the number of recovered DNA copies was quantified by real-time PCR using an Applied Biosystems 7500 real-time PCR thermocycler. The amplification products were visualized using gel electrophoresis (Lonza).

DNA amplification: To reduce cross-contamination during transfer, a removable PCR-tube for DNA collection was incorporated into the LabTube and it was used as a sample chamber in a modified LabReader. To run isothermal LAMP amplification and PCR inside the LabReader, temperature control as well as data analysis methods were implemented. A heated brass piece was fitted into the LabReader. It was heated using two parallel, electrically-insulated power resistors (Vitrohm 502-0;270\Omega) and a NTC (EPCOS, NTC B57540G1103F) was used as a temperature sensor. The NTC was connected to a serial resistor of 1.2k Ω , whose voltage was picked off from the temperature regulation module (Carel, IR33DIN). Temperature ramping was controlled using LabVIEW and executed by National Instrument modules. The achieved temperature was stable to $\pm 1.5^\circ\text{C}$. For LAMP amplification reagents from Mast Diagnostica and for PCR master mix from Qiagen (QuantiFast) were used. Both the LAMP and PCR reactions were visualized using the intercalating DNA dye SYTOX Orange.

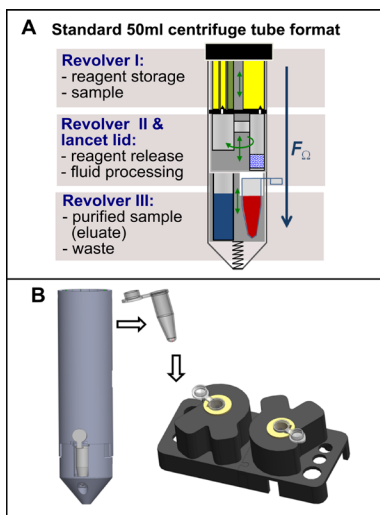


Figure 1. LabSystem workflow. (A) Layout of the LabTube and its 3 revolvers [5]. A removable PCR-tube for DNA collection was incorporated into revolver III. (B) After DNA purification, the PCR-tube containing the DNA was transferred into the temperature-regulated LabReader for amplification and readout.

Table 1. Sensitivity (S_n) and Specificity (S_p) of DNA purification and LAMP amplification for different applications inside the LabReader, in a real-time cyclers and compared with qPCR. For each sample $n > 20$ replicates were performed over at least 6 log scales from $0-10^9$ inserted copies (¹ ≥ 1000 copies, ² ≥ 100 copies, ³ ≥ 450 copies scales).

Samples	LabReader		LAMP in cyclers		qPCR	
	S_n (%)	S_p (%)	S_n (%)	S_p (%)	S_n (%)	S_p (%)
<i>E.coli</i> milk ¹	94	100	98	100	100	100
<i>E.coli</i> water ²	93	100	97	100	99	100
<i>Alicyclobacillus</i> juice ³	93	100	93	100	97	100

RESULTS AND DISCUSSION

DNA purification: Bacterial detection limit requirements in food safety are often low; hence purification kits yielding high efficiencies are required. DNA from *E.coli* lysate in milk and water, as well as from *Alicyclobacillus* lysate in apple juice was purified between 10^2-10^3 inserted copies in 45min using the standard protocol of the QiaAMP Micro DNA kit, which yielded the best performance of all screened kits. By optimizing the extraction protocol using 4 re-elutions, as little as 45 inserted copies were isolated (Fig.2).

DNA amplification: Since the detection of bacteria above a certain threshold limit often suffices, the qualitative LAMP DNA amplification was implemented into the LabReader. Bacterial DNA was amplified using a commercial VTEC *E.coli* LAMP assay with an intercalating DNA dye within 40min. The detection limits, LoDs^a, for both DNA purification and LAMP amplification were 10^2 and 10^3 copies of VTEC *E.coli* in water and milk and $4.5 \cdot 10^2$ copies for *Alicyclobacillus* in apple juice. Sensitivities and specificities were comparable with controls (Table 1). In order to allow for semi-quantification, real-time PCR was integrated into the LabReader. An intercalator-dye-based *E.coli* PCR was integrated. Using the same batch of reagents, a standard curve was created. The LabReader PCR had an efficiency of 95% and it was consistent with the real-time cyclers control. A melt-curve was performed after each run to differentiate specific from nonspecific products (Fig.3). The LoD^a of the PCR-based LabSystem was 10^2 and the quantification limit, LoQ^b, was 10^3 inserted copies. The data shown in Figure 3A was effectively acquired at 62°C . Unlike real-time cyclers, the LabReader reads out the signal continuously at all cycled temperatures. Hence, the signal from nonspecific products was eliminated by taking data at 85°C , which is above the melting-point of nonspecific products (Fig. 3C) [5]. The so achieved LoQ^b for the combined DNA purification, amplification and detection was reduced to 10^2 inserted copies for VTEC *E.coli* DNA from water and apple juice and 10^3 inserted DNA copies for VTEC *E.coli* from milk. This method simplifies data acquisition and analysis, as it eliminates the need to run a melting curve after each amplification reaction.

CONCLUSION

The automated LabSystem is low-cost and unlike many traditional and novel methods it is operated with standard equipment. The LabSystem does not require expert staff and it is flexibly usable for a variety of assay types (e.g. isothermal or PCR amplification). Unlike many commercially available benchtop DNA purification and amplification devices, it is not limited to specialized kits, it is easily scalable and reduces contamination risks through a standardized interface. It can be employed at small-to-medium throughput at e.g. the production site, at food services and sales locations. Its use is not limited to food safety. In the future, it could also be used for e.g. medical diagnostics, environmental contaminations and for quality control.

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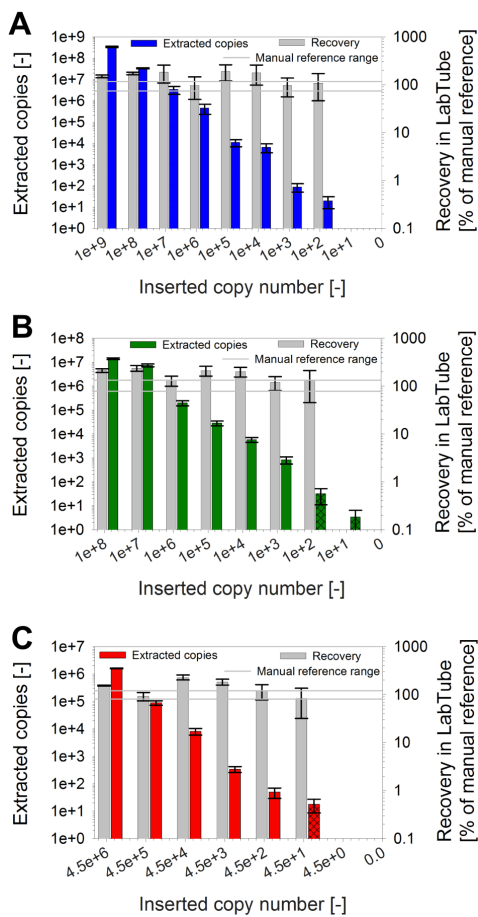


Figure 2. DNA purification in the LabTube for (A) *E. coli* in water (B) *E. coli* in milk (C) *Alicyclobacillus* in apple juice. The colored bars show the isolated DNA copies with the standard protocol (1 elution) and the hatched bars with 4 repeated elutions of the eluate. The yield in % of the manual reference is on average $156 \pm 38\%$.

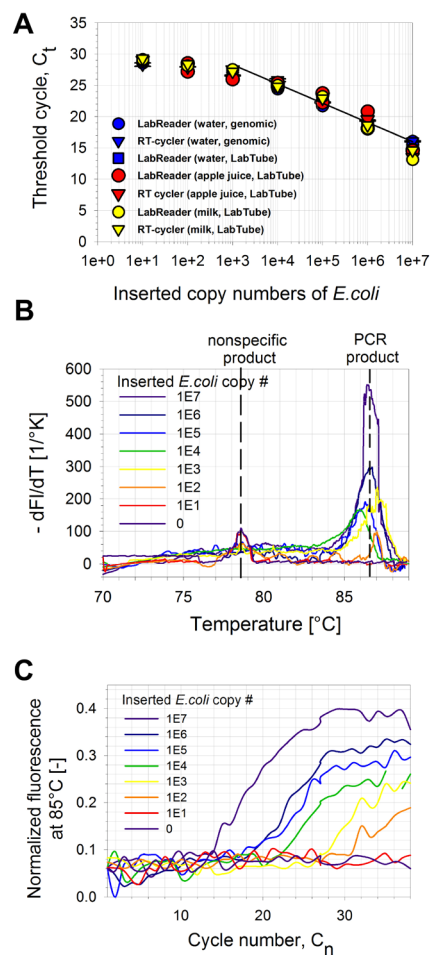


Figure 3. *E. coli* PCR in the LabReader using the intercalating DNA dye SYTOX Orange. (A) Threshold cycles, C_t , are shown for different copy numbers of *E. coli* lysates, which were isolated and purified from real samples using the LabTube. The readout temperature was 62°C in the LabReader. Results are compared with those of the real-time cycler. (B) The melting curve in the LabReader distinguishes PCR products at $T_{\text{melt}}=87^\circ\text{C}$ from nonspecific products at $T_{\text{melt}}=78^\circ\text{C}$. dF/dT is the negative change in normalized fluorescence with temperature. (C) The signal from nonspecific product is eliminated by reading out at 85°C , which is above the T_{melt} of the nonspecific and below the T_{melt} of the PCR products. The effective fluorescence is plotted vs. cycle number.

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^aLoD=Limit of Detection (3 SD above negative); ^bLoQ=Limit of Quantification (10 SD above negative).

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