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Oral

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

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Introduction:
Contamination of foods is a public health hazard that episodically causes thousands of deaths and sickens millions worldwide [1,2]. 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 [3,4]. To ensure food safety and quality, rapid, low-cost and easy-to-use detection methods are desirable. Here, the LabSystem is introduced for fully-integrated, automated DNA extraction and amplification. Recently, the basic mechanic principle of the LabTube, a disposable DNA extraction platform that runs in a laboratory centrifuge, was introduced [5]. Here, DNA extraction for *E.coli* and *Alicyclobacillus* from real samples (milk, juice and water) at $\geq 4.5 \cdot 10^1$ copies was established for the first time inside the LabTube. In the LabSystem, the extracted DNA was transferred into the LabReader, which consists of a low-cost, LED-based UV/Vis scheme [6]. The LabReader was modified to perform and analyze loop-mediated isothermal DNA amplification (LAMP) and PCR. Pathogenic DNA from real food samples was extracted and amplified at LOD[a]s$\geq 10^2$ using both methods.

Design:
To avoid cross-contamination during transfer, a removable PCR-tube for DNA collection was incorporated into the LabTube and 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. For PCR, temperature was regulated to ±1.5°C with LabVIEW using low-cost heaters (SMD resistors) and coolers (computer fans).

Results:
DNA Extraction: Bacterial detection limit requirements in food safety are often low; hence extraction 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 extracted to as low as 10^2 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 $\geq 4.5 \cdot 10^1$ inserted copies were extracted (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 *E.coli* VTEC LAMP assay with an intercalating DNA dye within 40min. The LoDs[a] for both extraction and LAMP amplification were $\geq 10^2$ and $\geq 10^3$ 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. Initially, an intercalator-dye-based *E.coli* PCR was integrated. Using the same batch of reagents, a standard curve was created. 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 $\geq 10^2$ and the LoQ[b]$\geq 10^3$ inserted copies. Additionally, signal from nonspecific products was eliminated by reading out signal at temperatures above the melting-point of nonspecific products.

Outlook:
The automated LabSystem is low-cost and does not require expert staff. It is suitable for small-medium throughput in e.g. food, medical and environmental applications. *(words: 500)*

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[a]LoD=Limit of Detection (3 SD above negative); [b]LoQ=Limit of Quantification (10 SD above negative).

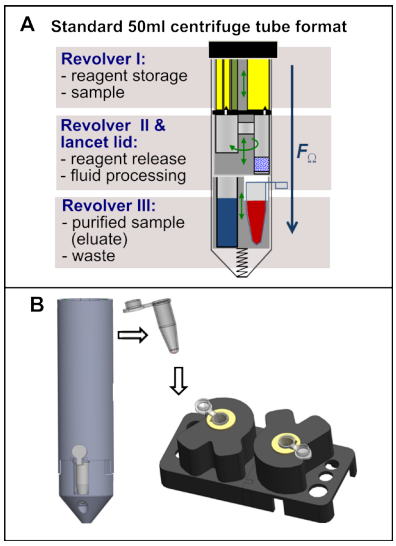

Figure 1: *LabSystem workflow.* ***(A)*** *Layout of the LabTube and its 3 revolvers [5]. A removable PCR-tube for DNA collection was incorporated into revolver III.* ***(B)*** *After extraction, the PCR-tube containing the DNA was transferred into the temperature-regulated LabReader for amplification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

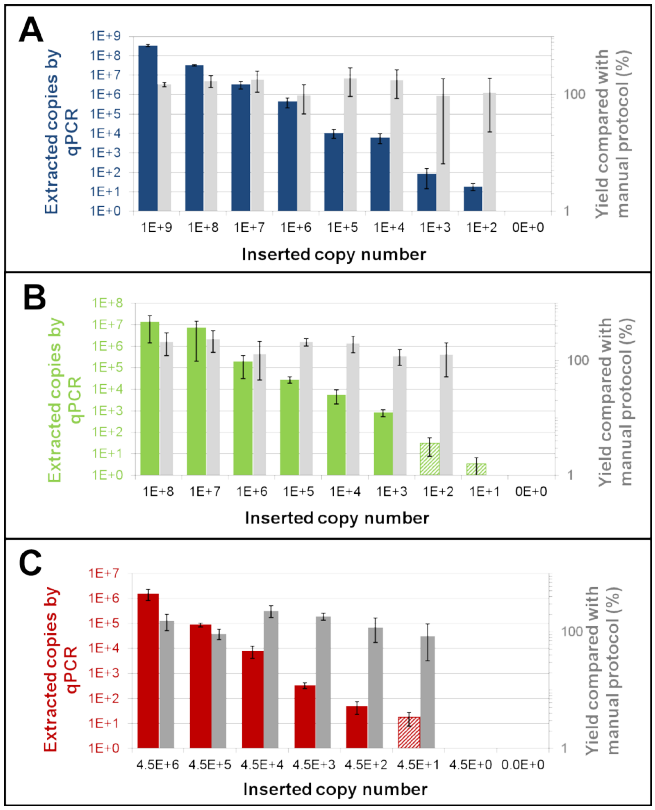

Figure 2: *DNA extraction in the LabTube for* ***(A)*** *E.coli in water* ***(B)*** *E.coli in milk* ***(C)*** *Alicyclobacillus in apple juice. The colored bars show the extracted DNA copies with the standard protocol (1 elution) and the hatched bars with 4 repeated elutions of the eluate. Grey bars show the yield in % of the manual reference, which on average is 156±38%.*

	LabReader		LAMP in cycler		qPCR	
Samples	**Sn (%)**	**Sp(%)**	**Sn (%)**	**Sp(%)**	**Sn (%)**	**Sp(%)**
***E.Coli* milk**[1]	94	100	98	100	100	100
***E.Coli* water** [2]	93	100	97	100	99	100
***Alicycloba-cillus* juice**[3]	93	100	93	100	97	100

Table 1: *Sensitivity (Sn) and Specificity (Sp) of extraction and LAMP amplification for different applications inside the LabReader, in a real-time cycler 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).*

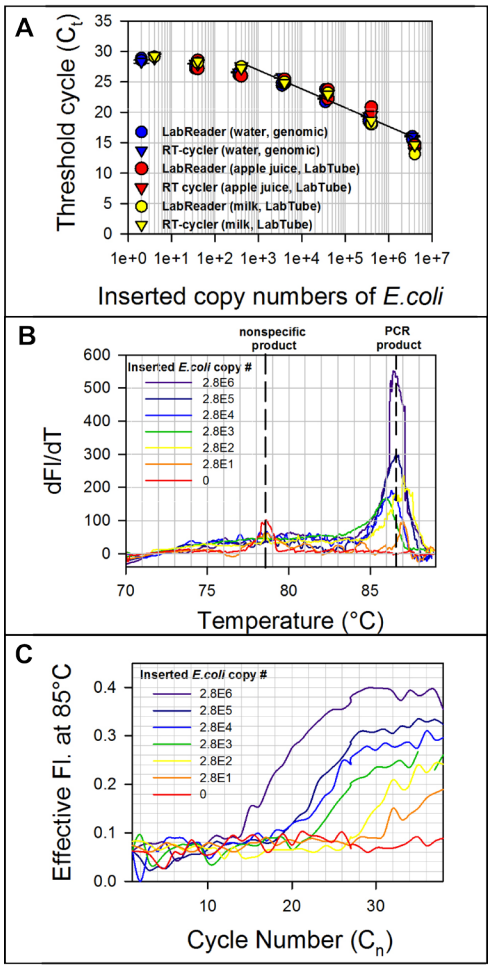

Figure 3: *E.coli PCR in the LabReader using the intercalating dye SYTOX Orange.* ***(A)*** *Threshold cycles, C_t, for different copy numbers of E.coli extracted from real samples with the LabTube are shown. The readout temperature was 62°C in the LabReader. Results are compared with those of the real-time cycler.* ***(B)*** *The melting curve in the LabReader distinguishes PCR products at T_{melt}=87°C from nonspecific products at T_{melt}=78°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.*