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) \textit{E.coli} are a major source of foodborne illness. Product spoilers, like \textit{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 \textit{E.coli} and \textit{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$^a \geq 10^2$ 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 $\pm 1.5 \degree C$ 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 \textit{E.coli} lysate in milk and water, as well as from \textit{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 \textit{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 \textit{E.coli} in water and milk and 4.5·$10^2$ copies for \textit{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 \textit{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$^b$ of the PCR-based LabSystem was $\geq 10^3$ 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.

**REFERENCES:**


\textsuperscript{a}LoD=Limit of Detection (3 SD above negative); \textsuperscript{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%.

**Figure 3:** E.coli PCR in the LabReader using the intercalating dye SYTOX Orange. (A) Threshold cycles, Ct, 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 Tmelt=87°C from nonspecific products at Tmelt=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 Tmelt of the nonspecific and below the Tmelt of the PCR products. The effective fluorescence is plotted vs. cycle number.

**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).