Analyst

Volume 1 | Number 1 | Jan 2013 | Pages 1–100

Analyst

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ARTICLE TYPE

Versatile-deployable bacterial detection system for food and environmental safety based on LabTube-automated DNA purification, LabReader-integrated amplification, readout and analysis

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Contamination of foods is a public health hazard that episodically causes thousands of deaths and sickens millions worldwide. To ensure food safety and quality, rapid, low-cost and easy-to-use detection methods
10 are desirable. Here, the LabSystem is introduced for integrated, automated DNA purification, amplification and detection. It consists of a disposable, centrifugally-driven DNA purification platform (LabTube) and the subsequent amplification and detection in a low-cost UV/vis-reader (LabReader). For demonstration of the LabSystem in the context of food safety, purification of *Escherichia coli* (non-pathogenic *E. coli* and pathogenic verotoxin-producing *E. coli* (VTEC)) in water and milk, and the
15 product-spoiler *Alicyclobacillus acidoterrestris* (*A. acidoterrestris*) in apple juice was integrated and optimized in the LabTube. Inside the LabReader, the purified DNA was amplified, readout and analyzed using both qualitative isothermal loop-mediated DNA amplification (LAMP) and quantitative real-time PCR. For the LAMP-LabSystem, the combined detection limits for purification and amplification of externally lysed VTEC and *A. acidoterrestris* is 10^2-10^3 cell-equivalents. In the PCR-LabSystem for
20 *E. coli* cells, the quantification limit is 10^2 cell-equivalents including LabTube-integrated lysis. The demonstrated LabSystem only requires a laboratory centrifuge (to operate the disposable, fully closed LabTube) and the low-cost LabReader for DNA amplification, readout and analysis. Compared with commercial DNA amplification devices, the LabReader improves sensitivity and specificity by the simultaneous readout of four wavelengths and the continuous readout during temperature cycling. The
25 use of a detachable eluate tube as an interface affords semi-automation of the LabSystem, which does not require specialized training. It reduces hands-on time from about 50 to 3 min with only two handling steps: sample input and transfer of the detachable detection tube.

Introduction

Contamination of foods is a public health hazard that episodically
30 causes thousands of deaths and each year sickens millions worldwide.[1,2] For example, verotoxin-producing *Escherichia coli* (VTEC) produce Shiga-like toxin, a main source of foodborne illness.[2,3] VTEC are oftentimes found in contaminated water, meat, dairy products and juice.[4] When infecting humans, they

have been linked with the severe complication haemolytic uremic syndrome.[2] For example, in 2012, 20 people got ill from drinking VTEC contaminated, unpasteurized milk in Oregon, USA[3] and in 1996, 66 people got sickened and one person died from VTEC
40 contaminated apple-cider.[5] 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 like *Enterococci* and *Campylobacter*.[6,7] Unlike pathogens, product spoiling bacteria do not cause sickness but great monetary losses to the
45 food industry. One prevalent product spoiler is *Alicyclobacillus acidoterrestris* (*A. acidoterrestris*) which is found in fruit juices, such as apple cider, and tomato products.[8,9] This endospore-forming bacterium is problematic to the juice industry because it survives pasteurization processes and because of its ability to
50 grow in low-pH environments without creating color or gas.[8,9]

To comply with food safety and quality regulations, the detection of small amounts of these bacteria is necessary. For

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example, within the European Union the permitted *E. coli* concentration of pasteurized milk is 100 CFU/ml[10] and that of milk for cheese production up to 10^4 CFU/ml.[10] The product spoiler *A. acidoterrestris*, on the other hand, causes off-flavor above 10^4 CFU/ml[11] and commonly requires limits of 1-5 CFU/10ml for quality control.[11] In order to comply with these regulations, samples are traditionally sent to specialized laboratories before the product is released or sold. In order to reduce transportation times and hence the time-to-result, testing methods are desirable that can be used by non-trained staff at the production site or sales location.[12-14] To detect relevant pathogens, pre-enrichment cultures or cell-plating and counting methods are commonly employed in testing laboratories. The time-to-result varies from days to weeks, which can cause economic losses, especially for perishable food products. Alternatively, PCR tests offer faster, more accurate test results than traditional microbiological culture methods. All described methods require scientific equipment (e.g. a thermocycler), a stable laboratory environment, a continuous refrigeration chain for reagents or antibodies, and/or specially trained staff to perform numerous manual steps[15-20], all of which are expensive and generally preclude their use at the production site or in the field.[19, 21] 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.[22] They are therefore not practical for low-throughput testing laboratories or sales locations, who cannot afford buying expensive automation equipment or employing specialized staff.[21]

Any automated diagnostic test for small-scale use should rely on an easier-to-use, cheaper and more robust technology.[12, 14, 19, 22] Thus, there has been an effort to develop low-cost, automated diagnostic biosensors, including nanotechnology-based sensors or microfluidic systems, such as droplet based[23-25], centrifugal[26, 27], capillary[12, 19, 28], pneumatic[14, 28, 29], paper-based[30-32] devices, and lateral flow assays. [12, 19, 20, 33] For the past decade, this has led to numerous publications about promising, early-stage technologies.[12, 19] Despite some commercially available systems in the field of medical diagnostics (e.g. Cepheid GeneXpert, Biocartis, and Abbott i-STAT), many of these systems still lack commercial maturity[33], especially when they include sample preparation steps.[19, 20] They further often require expensive hardware for optical readout or fluidic control and are usable for one kind of application only.[15, 19]

In this paper, we introduce the LabSystem, a semi-automated and frugal DNA purification, amplification and readout testing system for diagnostic and quality control applications (Figure 1). The LabSystem consists of the LabTube, a disposable cartridge for automation of DNA purification inside laboratory centrifuges, and the LabReader, a low-cost, handheld UV/Vis reader, to amplify, readout and analyze the purified DNA. The LabReader is low-cost, flexible and achieves high sensitivity and specificity by the simultaneous readout of four synchronous optical channels and continuous data readout during temperature cycling. Compared with manual reference methods, the LabSystem reduces hands-on time and it does not require specialized training. Purified DNA is collected in a LabTube-integrated, detachable PCR tube which can be directly transferred to the LabReader. This renders pipetting superfluous and reduces the risk of cross-contaminations. In this study, whole *E. coli* 477414 (*E. coli*) cells, as well as lysed VTEC and *A. acidoterrestris* in water, milk and juice were LabTube-purified, which represents the first extraction of bacterial cells inside the LabTube. As part of the LabSystem workflow, the purified DNA was amplified, readout and detected inside the LabReader and the results were compared with reference methods.

Materials and Methods

Detailed materials and methods may be found in the SI.

DNA purification

DNA was purified from known amounts of cells. *E. coli* (477414) was grown over night in LB medium (37°C) and *A. acidoterrestris* was grown in BAT medium at 37°C over night. Cell numbers were determined via cell-plating and counting. Heat inactivated VTEC (*E. coli* O157:H7) lysate was purchased from Biotecon Diagnostics GmbH, where the cell numbers were determined prior to inactivation via cell-plating and counting. Samples were processed with the QIAamp DNA Micro kit (Qiagen) according to the manufacturer's protocol. All processing steps were performed at room temperature and in a benchtop centrifuge of Hermle (Z326-K) with swing-bucket rotor. For automated processing, a centrifugation-time-protocol is transmitted via a computer interface (here, RS232) to the centrifuge.

Manual purification

100μl of fluid sample were mixed with 200μl AL buffer and carrier RNA and 20μl proteinase K. Next, 50μl ethanol (96%) was added and the mixture was vortexed for 5s. The mixture was transferred into a Mini Elute column, centrifuged at 6000g for 1 min and then washed twice with 450μl of AW1 buffer and then with 450μl of AW2 buffer. After drying the column at 6000g for 7min, the bound DNA was eluted with 20μl of elution buffer at 6000g for 1min.

LabTube purification

The fluid sample was loaded into revolver I that is prefilled with the above mentioned reagents AL buffer, proteinase K, Ethanol, AW1, AW2, and elution buffer. The LabTube was processed in a Hermle Z326K centrifuge. The purified DNA was collected in a removable PCR tube.

Quantification of recovered DNA by real-time PCR

After DNA purification, the number of recovered DNA copies was quantified by real-time PCR using an Applied Biosystems 7500 real-time PCR thermocycler. Every PCR reaction was conducted as triplicate for statistical significance. Compositions of the PCR reaction mixes and sequences of primers and probes are described in Table S-1, SI.

Control of PCR product by gel electrophoresis

The amplification products were visualized using gel electrophoresis (Lonza Flash Gel).

DNA amplification

LAMP amplification in LabReader

Blue and green LEDs (Cree, 5mm) were deployed as light sources. Light-voltage converters (TAOS, TSL257-LF) were used as detectors inside the LabReader. For filters, the following theater light filters (Rosco) were chosen: For the green LED "90
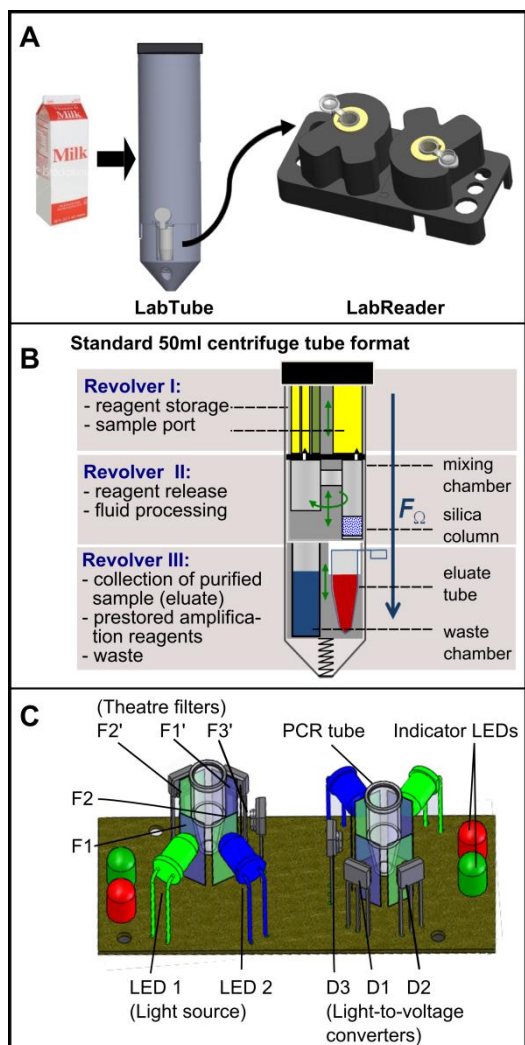
Fig. 1. LabSystem workflow. (A) DNA is purified inside the LabTube. The purified DNA is collected inside a LabTube-integrated PCR tube, which is transferred into the LabReader for amplification, readout and
5 analysis. (B) Schematic layout of the LabTube and its 3 revolvers for automated DNA purification. A removable PCR tube for DNA collection is incorporated into revolver III. (C) 3D-model of the LabReader without the black detector cover. UV light emitted by an LED (1 or 2) passes through an excitation filter (F1 or F2) made from low-cost theatre light
10 filters, the sample chamber, and emission filter (F1' or F2') before absorption is detected using a light-to-voltage detector (D1, D2). Fluorescence from both LEDs is detected at a third detector, D3 after passing through an emission filter F3'. Voltage outputs from the detectors (D1, D2, D3) are digitized and sent from a microcontroller to an external
15 computer. The indicator LEDs on the circuit board (red and green) tell the end-user whether the sample is contaminated or not.

dark yellow green" and for the blue LED "midnight blue" was selected as an excitation/emission filter; "amber red" was
20 integrated as a fluorescence emission filter. A heated brass piece was fitted into the LabReader and heated with a Minco foil (10Ω, HK5565R10.0L12F) and a negative temperature coefficient, NTC (EPCOS, NTC B57540G1103F), was employed as temperature sensor. The NTC was connected to a serial resistor of 1.2kΩ,
25 whose voltage was picked off from the temperature regulation module (Carel, IR33DIN). For LAMP amplification an Isoplex VTEC screening kit (Mast Diagnostica) was used. For

A. acidoterrestris a primer set (Eiken Chemicals) was used in combination with a DNA amplification kit (Mast Diagnostica).
30 For detection 0.2 μM SYTOX Orange dye and Lucifer Yellow were chosen as a passive reference (Life Technologies). In the LabReader 20μl of master mix (pre-stored in liquid form inside the detachable PCR tube) and 20μl of sample were processed. A LAMP control was always run in parallel in a real-time cycler
35 with 10μl of master mix and 10μl of sample.

PCR in LabReader
The LabReader was heated using two parallel, electrically-insulated power resistors (Vitrohm 502-0;270\Omega) connected in parallel. The sensors were cooled using a fan (NMB-MAT,
40 1606KL) each from the top of the setup. Temperature ramping was controlled using Lab VIEW and executed by National Instrument modules. Mineral oil (Sigma Aldrich) was added on top of the PCR mix, in order to avoid evaporation to the top of the PCR tube. A melt-curve was run as a control to differentiate
45 the product from nonspecific products, such as primer dimers.

Detection and quantification limit

The detection limit, LoD, was determined according to the International Conference on Harmonization (ICH) standards, which defines the LoD as 3 standard deviations of the negative
50 control, implying that the probability of false positive is small (1%) and that of a false negative is 50% for a sample that has a concentration at the LoD.[34, 35] The limit of quantification, LoQ, was calculated as 10 standard deviation of the negative control.[34, 35]

55 ## Results

Concept of the LabSystem workflow

In the LabSystem workflow, DNA is purified inside the LabTube and it is collected inside a removable PCR tube that contains pre-stored DNA amplification reagents. For amplification, the
60 removable PCR tube is manually transferred from the LabTube to the low-cost LabReader, which affords fully automated DNA amplification, readout and analysis (Figure 1A). Compared with the manual reference, only one instead of 13 pipetting steps and only one instead of five centrifuge (un-)loading steps is
65 necessary, hence reducing the hands-on time from 50 to 3 min. Due to the semi-automation of the system, the risk of manual pipetting errors and that of cross-contamination is substantially reduced.

DNA purification in the LabTube
70 The LabTube is a microfluidic cartridge for automated DNA purification inside laboratory centrifuges.[36] It is based on a disposable cartridge with the dimension of a 50ml centrifuge tube, as shown in Figure 1B. Controlled by an assay-specific centrifugation-time protocol, integrated unit operations for
75 reagent addition, mixing and solid-phase extraction enable automated processing of the complete DNA purification workflow. 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
80 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
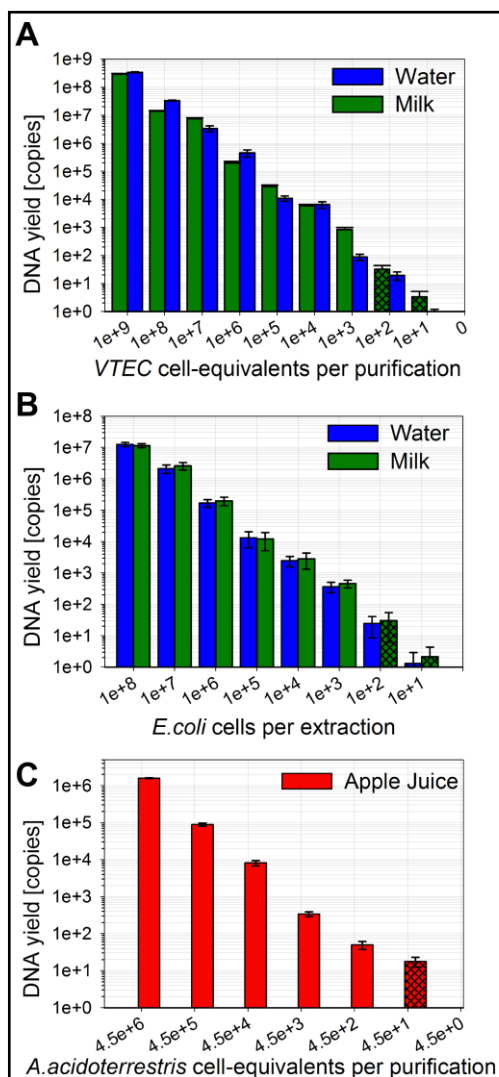
Fig. 2. DNA purification in the LabTube for (A) VTEC (O157:H7) lysate in water and milk (B) *E. coli* (477414) cells in water and milk (C) *A. acidoterrestris* lysate in apple juice. The colored bars show the purified DNA copies with the standard protocol (1 elution) and the hatched bars with 4 repeated elutions of the eluate. 100μl of sample were processed in each LabTube.

separation. The required centrifugation-time-protocol is transferred to the centrifuge via a computer interface (here, RS 232). Unlike other systems for automated DNA purification, the LabTube requires only a laboratory centrifuge, it is fully closed (reducing contamination risks) and it offers flexibility for adaption to automate a variety of other assay protocols. In this paper, to reduce pipetting steps and hence cross-contamination risks during transfer, a removable, low-cost PCR tube was incorporated into the LabTube for DNA collection (Figures 1A and S18). The detachable PCR tube is also used as a sample chamber inside the LabReader.

LabReader for DNA amplification, readout and analysis

For DNA amplification, readout and analysis we used a multichannel, multisample, UV−vis spectrophotometer/ fluorometer that employs two frequencies of light simultaneously to interrogate the sample. The LabReader is based on a round geometry[37, 38], in which two LEDs serve as UV and fluorescence

light sources to readout four different wavelengths simultaneously. Figure 2C shows a schematic overview of the LabReader optics. UV-light emitted by a green LED 1 passes through an excitation filter (F1) made from low-cost theatre light filters, the sample, and emission filter (F1') before absorption is detected with a light-to-voltage detector (D1). Light from blue LED 2 is filtered prior (F2) and after (F2') passing through the sample chamber to the detector, D2. FAM and ROX fluorescence from LEDs 1 and 2 is detected at a third detector, D3, after passing through fluorescence emission filter F3'. Voltage outputs from the detectors (D1, D2, D3) are digitized and sent from a microcontroller to an external computer, tablet or cell-phone. The LED light-outputs are stabilized at a constant level using low-cost light-to-voltage detectors and op-amps arranged in a feedback loop[37, 38] (Figure S7). The indicator LEDs on the circuit board (red and green) tell the end-user whether the sample is contaminated or not. As indicated in Figure 2A and S7, the optics is encased in a light-proof housing made of stereolithography. To assemble a device, two mirror-image enclosure units are snapped together and placed over the circuit board containing the LEDs and detectors (Figure S7). The optical setup and electronics are aligned in the cover by springs inside the enclosure, which force the parts against reference components. In this study, temperature control units, as well as automated data analysis methods were developed and integrated into the LabReader, in order to enable automated DNA amplification, readout and analysis (see SI). Further, the LabReader was made compatible with a plastic PCR tube as a sample chamber rather than with a glass cuvette (Figure S-8) and it can be operated with a reduced sample volume of ≥40μl to minimize reagent costs.

Compared with commercial DNA amplification devices, the LabReader can readout four wavelengths simultaneously and it continuously reads out data during the DNA amplification temperature cycling, which affords the potential for improved sensitivity and specificity. Due to the use of theater light filters, LEDs and light-to-voltage converters as detectors, the LabReader is expected to be lower in cost than traditional laboratory equipment, whilst achieving similar detection limits (as shown in this study).

DNA purification results

LabTube DNA purification

For evaluation of the DNA purification performance of the LabTube compared with the manual reference, DNA from *E. coli* cells in milk and water, as well as from *A. acidoterrestris* lysate in apple juice was purified. For *E. coli*, whole cells of a non-toxic strain (41447), as well as cell lysates of toxic VTEC (O157:H7) were purified using the QIAamp DNA Micro kit. As indicated by the solid bars in Figures 2A and B, the detection limit of LabTube purified DNA from *E coli* cells and VTEC lysate is 10^2 inserted cell-equivalents in water and 10^3 inserted cell-equivalents in milk (detected by qPCR). For *A. acidoterrestris* lysate in apple juice, the LabTube-purification limit is 10^3 inserted cell-equivalents per LabTube purification, as indicated by the solid bars (Figure 2C). The purified cell-equivalents are above the LoD of the qPCR system (20 cell-equivalents/extraction; see Figure S-5 and S-6) and could therefore be determined with more than 95% confidence. In these experiments, 100μl sample was processed, even though up to 4ml can be purified per LabTube run. The

LabTube standard errors at each concentration are on average 15%, which is comparable with those of the manual reference (see SI). The DNA purity inside the LabTube ($A_{280}/A_{260}=1.92\pm0.11$) is comparable with the manual reference ($A_{280}/A_{260}=1.89\pm0.08$). Using the LabTube, DNA from *E. coli* and *A. acidoterrestris* lysates was purified at an efficiency of $157\pm21\%$ compared with the manual reference, which was normalized to $100\pm17\%$ (averaged over all measured concentrations). DNA from whole *E. coli* cells was extracted inside the LabTube with an efficiency of $91\pm17\%$ compared with the manual reference. For DNA extracted from cell lysates, the higher efficiency inside the automated LabTube might be explained by the better mixing efficiency of ethanol with cell lysate inside the LabTube compared with the manual reference. For the purification of DNA from whole cells including lysis, it is expected that the reduction in efficiency is caused by a less efficient lysis step inside the LabTube than for the manual reference. It is assumed that the integrated mixing system of the LabTube, which is based on the inversion of liquid layers[36], is more efficient for mixing ethanol with water-based mixtures (binding step) than two liquids of similar densities (lysis step). Further, the integrated mixing system mixes components macroscopically only, which may be sufficient for the binding step but not equally effective for the lysis step. This effect is observed to be more predominant for gram-positive ($65\pm15\%$ for whole *A. acidoterrestris* cells) than for gram-negative cells.

Optimization by multiple elutions

Even though LabTube purifications have similar efficiencies as the manual reference, only around one to two tenth of the inserted DNA is recovered in both cases (Figure 2). To increase the recovery (i.e. the percentage of purified compared with inserted cell-equivalents), optimization of the manual protocol was performed. The goal was to further reduce the purification detection limits inside the LabTube. Whilst elevated lysis temperature and multiple binding of the eluate do not significantly affect the recovery (Figures S2 and S3), re-eluting the eluate 4 times increases the recovery from $11\pm7\%$ to $56\pm21\%$ of the inserted cell-equivalents (Figure S-4). The result was verified inside the LabTube: The hatched bars in Figure 2 indicate that four repeated elutions of the eluate reduces the purification detection limit by an order of magnitude: for *E. coli* cells and lysed VTEC in milk, it is 10^2 cell-equivalents, and for *A. acidoterrestris* lysates in apple juice it is $4.5\cdot10^2$ cell-equivalents per LabTube-purification (here, 100µL of sample was used). The standard error (33%) is comparable to that of single elutions at low concentrations (32%). In the future, when lower detection limits are needed, multiple elutions should be automated inside the LabTube. For the applications covered in this study, single elutions were sufficient.

Bacterial DNA amplification and detection

LAMP-LabSystem

In the LAMP-LabSystem, DNA is purified with the LabTube, transferred via a removable eluate-tube and isothermally (LAMP) amplified, readout and analyzed using the LabReader.

In conjunction with fluorescent, intercalating DNA dyes (rather than turbidity readout), the isothermal loop-mediated

Table 1. Loop-mediated isothermal DNA amplification (LAMP) of LabTube-purified bacterial DNA. The percentage of positive reactions is shown for LAMP reactions inside the LabReader, for the LAMP reaction control in the real-time cycler and for the qPCR control. The results are shown for different concentrations of DNA in the LabTube eluate for VTEC and *A. acidoterrestris* in water, milk and juice.

Sample and inserted cell-equivalents into the LabTube		LAMP LabReader Positive reaction (%)	LAMP rt-cycler Positive reaction (%)	qPCR rt-cycler Positive reaction (%)
VTEC from water	10^9	3/3	5/5	5/5
	10^8	3/3	5/5	5/5
	10^7	3/3	5/5	5/5
	10^6	3/3	5/5	5/5
	10^5	3/3	5/5	5/5
	10^4	3/3	5/5	5/5
	10^3	3/3	9/10	9/10
	10^2	2/3	9/10	10/10
	10^1	1/3	2/10	0/10
	0	0/3	0/10	0/10
VTEC from milk	10^8	3/3	5/5	5/5
	10^7	3/3	5/5	5/5
	10^6	3/3	5/5	5/5
	10^5	3/3	5/5	5/5
	10^4	3/3	5/5	5/5
	10^3	2/3	9/10	9/10
	10^2	0/3	2/10	0/10
	10^1	0/3	0/10	0/10
	10^0	0/3	0/10	0/10
	0	0/3	0/10	0/10
***A. acidoterrestris* from apple juice**	$4.5\cdot10^6$	3/3	5/5	5/5
	$4.5\cdot10^5$	3/3	5/5	5/5
	$4.5\cdot10^4$	3/3	5/5	5/5
	$4.5\cdot10^3$	3/3	5/5	5/5
	$4.5\cdot10^2$	2/3	8/10	5/5
	$4.5\cdot10^1$	1/3	3/10	0
	$4.5\cdot10^0$	0/3	0	0
	0	0/3	0	0

Table 2. Summarized sensitivity (Sn) and specificity (Sp) of the LAMP-LabSystem in different applications. The values were calculated from LabTube-purified samples shown in Table 1. (for sensitivity calculations: [1]$\geq10^2$ cell-equivalents, [2]$\geq10^3$ cell-equivalents, [3]$\geq4.5\cdot10^2$ cell-equivalents before the purification).

Samples	LabReader		LAMP in rt-cycler		qPCR	
	Sn (%)	Sp(%)	Sn (%)	Sp(%)	Sn (%)	Sp(%)
VTEC water[1]	93	100	97	100	99	100
VTEC milk[2]	94	100	98	100	100	100
***A. acidoterrestris* juice**[3]	93	100	93	100	97	100

DNA amplification reaction (LAMP) is of qualitative nature.[39] Since in many cases the desired test result does not have to be quantitative, but instead the presence or absence above a certain threshold suffices, a LAMP reaction was integrated into the LabReader. By employing a LAMP instead of a PCR reaction, the overall time for the amplification is reduced from 1-2hrs to 40min, hence allowing timely and goal-directed decision making.[40] Unlike PCR, LAMP does not require thermal cycling[41-43], allowing a simpler and cheaper, disposable heating system to be used[44-48], and it is particularly temperature robust[40, 48, 49]. We observed the LAMP reaction to be stable at temperatures of

65±5°C (Table S-6). In order to control the LabReader temperature at 65°C, a metal (brass) insert was fitted into its housing, which was heated with a heating foil (Minco) and regulated with a control module (Carel) and a temperature sensor [5] (NTC), achieving temperature stabilities of ±1.5°C (SI 2.2; Figure S-9). LAMP amplification was performed with commercially available detection kits (Mast/Eiken) and visualized with the intercalating DNA dye SYTOX Orange. Due to the use of two LEDs and four synchronous channels in the [10] LabReader, normalization by a passive reference dye, Lucifer Yellow, was performed. Dye-compatible theatre light filters and LEDs were incorporated into the frugal LabReader. Fluorescent signals were recorded over time via a USB port and analyzed and displayed on a laptop (see SI 2.2). Gel electrophoresis confirmed [15] the reaction products (Figure S-11) and controls were run in a real-time cycler in parallel.

The LAMP-LabSystem workflow consists of DNA purification inside the LabTube, collection of the purified DNA in a detachable detection tube (which contains pre-stored LAMP [20] reagents), followed by DNA amplification, readout and analysis inside the LabReader. Table 1 shows the results of complete LAMP-LabSystem workflows, including controls, for VTEC and *A. acidoterrestris* lysates over at least 6 log-scales. The LoDs for both purification in the LabTube and LAMP amplification in the [25] LabReader are 10^2 and 10^3 VTEC cell-equivalents in water and milk and $4.5 \cdot 10^2$ *A. acidoterrestris* cell-equivalents in apple juice (data were not interpolated). The overall time-to-result for both purification and amplification in the LAMP-LabSystem is ~90min (50min for DNA extraction including lysis, 1-2 min for [30] eluate transfer and 40min for LAMP amplification and readout). Above the LoDs for VTEC and for *A. acidoterrestris*, the average sensitivity (probability of a true-positive result) is 93±1%. The specificity (probability of a true-negative result) is 100% (0/9 experiments). Sensitivities and specificities are comparable with [35] controls in the real-time cycler (Table 2). In addition, the values are comparable with those of qPCR (Table 2) and they are consistent with literature values.[41, 43] The achieved detection limits of the LAMP-LabSystem are similar to commercially available rapid detection methods for food samples, which vary [40] between 10^2-10^4 CFU/ml for most PCR-based systems.[11, 50]

PCR-LabSystem

In order to allow for semi-quantification, real-time PCR was integrated into the LabReader. As a first example, a PCR reaction for *E. coli* was integrated and visualized using the intercalating [45] DNA dye, SYTOX Orange. In order to run a PCR reaction in the LabReader, temperature ramping cycles were incorporated. The LabReader was heated using two electrically insulated power resistors connected in parallel (270Ω) inside a metal-fitting required for temperature stabilization (Figure S-12; SI 2.3). The [50] optical detection chambers were each cooled with a fan from the top of the setup (Figure S-13). Temperature ramping was controlled using Lab VIEW and executed by National Instrument modules. The achieved temperature profile is shown in Figure S-13B. Fluorescence values were readout as voltage values via a [55] USB port from the LabReader and automatically analyzed (Figure S-14). The threshold cycle, C_t, was defined as the cycle at which the average fluorescence signal within the first ten cycles had increased by 15% (see SI 2.3).[51]

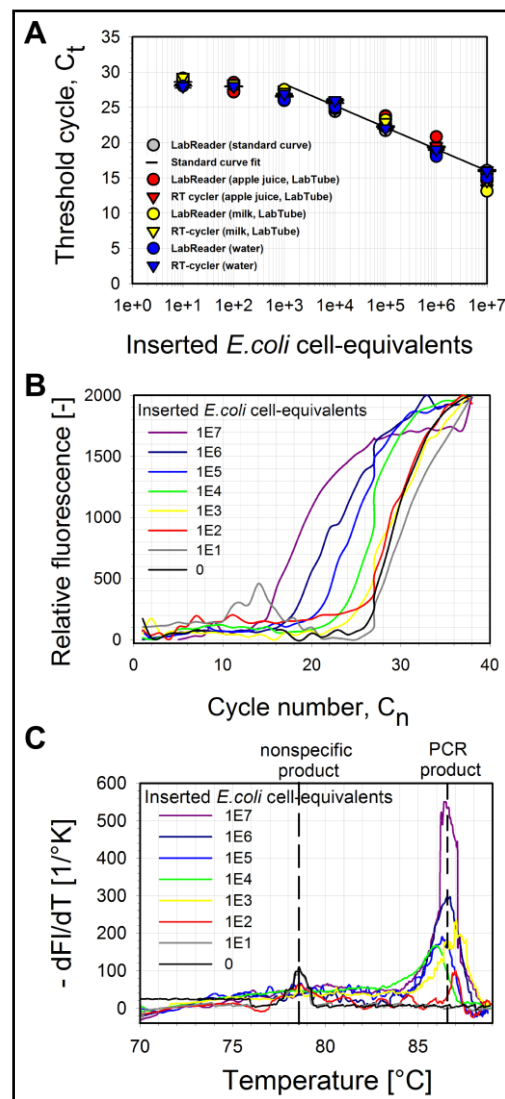To verify the PCR reaction inside the LabReader, a standard

Fig. 3. *E. coli* PCR in the LabReader using the intercalating dye SYTOX [60] Orange. **(A)** Threshold cycles, C_t, for different inserted copy numbers of *E. coli*, which were previously purified from real samples using the LabTube. ***The readout temperature was 62•°C.*** **(B)** Reaction curves for different *E. coli* cell-equivalents in water (fluorescence relative to the [65] average of the first ten cycles vs. cycle number). **(C)** The melting curve distinguishes PCR products at T_{melt}=89°C (solid line) from nonspecific products at T_{melt}=78°C (dashed line). dF/dT is the change in fluorescence over temperature.

curve of known amounts of genomic *E. coli* DNA was created [70] (grey dots in Figure 3A). The standard curve consists of a log-dilution series of 10^6 *E. coli* genomic DNA copies, which according to Figure 2A corresponds to $1.1 \cdot 10^7$ cell-equivalents inserted into the LabTube (x-axis). Because the same batch of reagents was used, semi-quantification was possible (for batch-[75] independent quantification at least four controls are required[52, 53]). A control was run in the real-time cycler. A fit revealed that above 10^3 corresponding, inserted cell-equivalents (on the x-axis) the PCR reaction has an efficiency of 110% in the real-time cycler and 95% in the LabReader (black line), which is within the [80] acceptable literature range[51]. The lower efficiency in the LabReader is likely due to variations in cycle times (~310±20s), as well as the temperature inaccuracy of ±1°K in the LabReader.
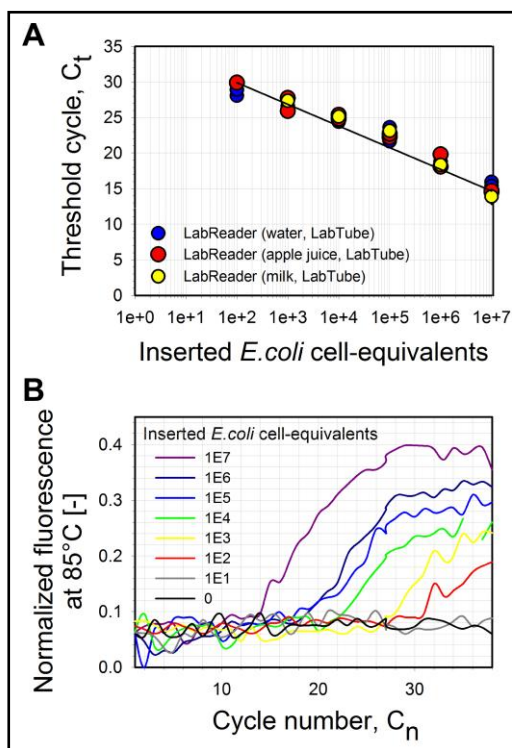
Fig. 4. *E. coli* PCR in the LabReader using the intercalating dye SYTOX Orange with ***readout at 85°C*** above the melting point of nonspecific products **(A)** The calibration curve no longer shows false positive signals below 100 cell-equivalents. **(B)** Reaction curves for different copy numbers of genomic *E. coli* DNA in water (effective fluorescence vs. cycle number). Normalized fluorescence is the difference in fluorescence signal between 85°C and 95°C divided by the difference in fluorescence signal between 62°C and 95°C at each cycle number.

For the standard curve, the average standard error in the LabReader is ±11% (corresponding to a threshold cycle variation of C_t ±0.15), whereas it is only ±5% (corresponding to C_t±0.08) in the real-time cycler.

This difference is expected to be caused by consecutive rather than parallel sample-processing inside the LabReader. As indicated in Figure 3A, the calibration curve is only linear above 10^3 corresponding, inserted cell-equivalents (x-axis), which is attributed to the presence of primer dimer reactions below this limit as confirmed by gel electrophoresis (Figure S-16). To differentiate specific from nonspecific products, a melting curve of the reaction products hence needs to be performed below 10^3 inserted cell-equivalents.

After establishing the standard curve, the complete PCR-LabSystem workflow was performed and compared with a real-time cycler control. Here, different concentrations of *E. coli* in water, milk and apple juice were extracted using the LabTube. The DNA was collected in a removable PCR tube containing pre-stored PCR reagents and it was then automatically amplified, readout and analyzed inside the LabReader. Figure 3A depicts the results from the LabReader (colored round dots) and real-time cycler (triangles). Strikingly, all data for the LabReader fall onto a universal master curve. The overall LoD for both purification and amplification in the PCR-LabSystem is 10^2 and the LoQ $2·10^3$ (interpolated) inserted *E. coli* cell-equivalents from water and apple juice. For *E. coli* from milk, the LoD is 10^3 and the

LoQ is $2·10^4$ inserted cell-equivalents. Up to 4ml of sample can be processed per run. The overall time-to-result for DNA extraction, amplification and readout inside the PCR-LabSystem is ~160min (50min for DNA extraction including lysis, 1-2 min for eluate transfer and 110min for the PCR reaction and readout). To differentiate specific from nonspecific products, a melting curve of the reaction products was performed (Figure 3C). For the complete LabSystem workflow, the lack of PCR products was observed below 10^2 inserted cell-equivalents and the presence of primer dimers at or below 10^3 inserted cell-equivalents (Figure 3C).

The data shown in Figure 3 was effectively acquired at 62°C. Because the nonspecific product melts at 78°C, the signal from both the nonspecific and specific PCR products is detected at 62°C. Unlike the real-time cycler, the LabReader reads out the signal continuously at all temperatures and the temperature is plotted along with the amplification data. It was hence hypothesized that signal from nonspecific primer dimers could be eliminated by reading out data above the melting temperature of the nonspecific product (78°C) and below that of the PCR product (87°C).[54] The acquired data were reanalyzed and the normalized slope was plotted at 85°C (see SI 2.3.2). Using this method, the signal from nonspecific product was eliminated and a linear calibration curve was created (Figure 4). The LoQ for the complete PCR-LabSystem workflow is 10^2 inserted *E. coli* cell-equivalents from water and apple juice and 10^3 inserted *E. coli* cell-equivalents from milk. The standard error of C_t±0.18, i.e. 15.3%, is comparable with that from the readout at 62°C (C_t±0.15, i.e. 11%) shown in Figure 3. This readout method greatly simplifies data acquisition and analysis, as it eliminates the need to run a melting curve. The described readout option is not easily incorporated into a traditional real-time cycler without adding an additional readout step of several seconds to the temperature profile (e.g. 85°C for 20s/cycle). This additional step both elongates the run and alters the temperature profile, which could affect results. The continuous data readout, which does not require altering the temperature profile, is therefore a real advantage of the LabReader.

Sample concentration and pre-enrichment.

The achieved detection and quantification limits imply that no pre-enrichment is needed for many food safety applications of e.g. *E. coli*, where the required LoD in pasteurized milk or milk during cheese production is 10^2-10^4 CFU/ml or when the spoilage of *A. acidoterrestris* in juice (10^4 CFU/ml) needs to be detected.[10, 11] When lower detection limits or larger testing volumes are needed, sample concentration or pre-enrichment steps are necessary[55]. Commercially available centrifugal filters afford the concentration of samples (e.g. Sartorius Vivaspin). Additionally, instead of using an expensive incubator often not available in the field, the heated LabReader can be employed for pre-enrichment. Pre-enrichment is also useful for live-dead discrimination at low bacteria concentrations: Standard DNA amplification methods do not differentiate viable from dead cells, which is particularly relevant for low cell numbers in heat-inactivated sample matrices. Figure S-17 depicts that *E. coli* cells are pre-enriched from 10 CFU/ml to 10^4 CFU/ml within 4 hours inside the LabReader. For live-dead discrimination, this step could be coupled to a raised cutoff for positive PCR readings or a

comparison of C_t values prior and after pre-enrichment.

Discussion and outlook

An integrated, low-cost and semi-automated DNA purification, amplification and readout system, the LabSystem, was introduced. The system consists of the disposable LabTube cartridge that automates manual steps involved in DNA purification inside a laboratory centrifuge; and a low-cost, optical, LED-based UV/Vis reader, the LabReader, for fully-automated amplification, detection and analysis of the purified DNA. In the combined LabSystem workflow, the extracted DNA is collected inside the LabTube in a PCR tube containing DNA amplification reagents and it is transferred into the LabReader. Inside the here introduced LabReader, the purified DNA is automatically amplified, detected and analyzed via both the qualitative, isothermal LAMP reaction (LAMP-LabSystem) and the semi-quantitative real-time PCR (PCR-LabSystem). The product specificity is determined in the PCR-LabSystem by performing a melting curve or by data readout at temperatures above their melting point (here 85°C). The combined purification and amplification LoD of the LAMP-LabSystem is 10^2-10^3 inserted cell-equivalents of VTEC in water and milk and *A. acidoterrestris* in apple juice with a time-to-result of 90min. The combined purification and amplification LoQ of the PCR-LabSystem is 10^2 inserted cell-equivalents for *E. coli* in water and juice and 10^3 inserted cell-equivalents in milk at a readout temperature of 85°C, with a time-to-result of 160min. The results are comparable with commercially available rapid detection methods (e.g. PCR-based) in food samples.[55] The achieved detection limits for LAMP and real-time PCR imply that no pre-incubation is needed for many food safety applications (e.g. *E. coli* in water or milk products, with an LoD in production processes of 10^2-10^4 CFU/ml[10]). When lower detection limits are required or for live-dead discrimination, pre-enrichment in the LabReader can be employed.

The LabTube as a component of the LabSystem is easily scalable: this refers to the sample volume that can be processed within one LabTube as well as the fact that up to 20 LabTubes can be processed simultaneously within one centrifuge. It minimizes contamination risks by being a fully-closed system and by the here introduced PCR tube as an interface to the LabReader. The LabReader affords flexibility (it can be controlled by a tablet or phone) and versatility (by enabling the readout of four simultaneous wavelengths, as well as the performance of both isothermal (here LAMP) and PCR DNA amplification). These attributes make the LabReader usable for a variety of targets and assay types. The LabReader's ability to readout data continuously during temperature cycling (without altering the temperature profile) enables improved data analysis (e.g. the elimination of a melt curve) compared with a traditional real-time cycler. The LabReader can be easily parallelized, it is flexible and it is expected to be lower in cost than commercially available real-time cyclers due to the use of LEDs and theater light filters.

The semi-automated LabSystem workflow substantially reduces pipetting steps and hence cross-contamination risk. Compared with manual methods, the hands-on time for the LabSystem is reduced from 50 to 3 min by only requiring two instead of 13 manual steps. Compared with fully automated, commercial systems (e.g. the market standard GeneXpert from Cepheid), the LabSystem currently has four instead of six (GeneXpert[56]) optical channels and multiplexing yet has to be demonstrated. Compared with fully-automated systems, the semi-automated LabSystem has a higher contamination risk. However, the here introduced removable PCR tube limits this risk inside the LabSystem and it makes it more flexible than fully-automated systems, like the GeneXpert: the semi-automation affords the separation of the used DNA extraction and amplification systems or the storage of the purified DNA prior to further processing. The LabReader is also more flexible than the GeneXpert[56], as both PCR, LAMP and other biochemical reactions can be used without being restricted to specialized kits. The LabSystem performs proper DNA purification (which the GeneXpert does not[56]), hence making it less susceptible to PCR-inhibitors from difficult-to-extract matrices. Most importantly, the LabSystem is ultimately expected to be an order of magnitude lower in cost than the GeneXpert[56] and other commercial systems, whilst having a similar throughput.

The achieved results can be extended and improved in a variety of ways. In its current form, the LabSystem (as well as most commercial systems) is deployable in small laboratories, at production sites or quality control centers that have a stable, uninterrupted power supply and access to a laboratory centrifuge. To make the LabSystem more broadly deployable, a lower-cost or battery-driven LabTube processing device should be developed and the LabReader should be run on batteries.

In its current form, it is expected that the LabSystem is suitable for the detection of a broad range of bacteria, such as lactic acid bacteria in e.g. juices and *bacillus cereus* and *clostridium perfingens* in milk, which have safety limits between 10^4-10^7 CFU/ml.[10] In the future, the LabSystem could be used to monitor bacteria in other matrices that have not yet been purified with the LabTube. This includes e.g. bacteria in sewage purification plants and in pharmaceutical fermentation processes. When lower detection limits are needed, we suggest to insert larger sample volumes (up to 4ml instead of the 100µl used in this paper) as well as to automate multiple elution steps in the LabTube, which were demonstrated to reduce the purification detection limit by an order of magnitude. For live-dead discrimination at low bacteria concentrations, pre-enrichment inside the LabReader was demonstrated in this study. As a more rapid alternative to pre-enrichment in the future, staining methods in conjunction with PCR (e.g. ethidium bromide monoazide, EMA[57], or propidium monoazide, PMA[58]) could be employed. The photoactivable dyes EMA and PMA only penetrate dead cells with compromised membrane/cell wall systems.[57, 59] DNA covalently bound to the dye cannot be PCR amplified, thus only DNA from viable cells is detected by PCR. In the introduced LabSystem, photoactivation of the dyes could be performed inside the LabReader and dye removal prior to cell lysis could be automated inside the LabTube.[57] Alternatively, viable and dead cells could be differentiated inside the LabSystem by purifying mRNA (instead of DNA) and by detecting it via reverse transcriptase PCR.[57, 59]

To allow for batch-independent quantification during DNA amplification inside the LabReader, more than four reaction

chambers should be parallelized.[52, 53] A third LED could be added to each chamber to detect six instead of four parallel optical channels. In order to increase sensitivity and specificity of the PCR reaction, target-specific probes and multiplexing assays should be implemented.

Conclusions

The LabSystem has been introduced for the purification, amplification and detection of $\geq 10^2$ *E. coli* cells, VTEC and *A. acidoterrestris* cell lysates in food matrices (milk, apple juice and water). The combined LabSystem can be used without specialized training and only requires a laboratory centrifuge and the low-cost, versatile LabReader. The LabSystem only requires two processing steps and a hands-on time of 3min, which represents a one order of magnitude reduction compared with manual systems. The frugal, automated LabSystem is flexibly deployable for a variety of applications and assay types (e.g. isothermal LAMP or PCR amplification) and it affords improved data analysis (four simultaneous channels and continuous data readout during temperature cycling). Unlike many commercially available benchtop purification and amplification devices, it is easily scalable (up to 4ml sample and up to 20 parallel runs), it reduces contamination through a standardized interface (detachable detection tube) and it is not limited to specialized kits or assays.

The combined LabSystem could help hastening more testing and analysis at the location of an outbreak, the production site or at the point-of-care. Ultimately, this could increase safety, reduce contamination outbreaks, as well as the waste of precious resources in food applications, but also in other areas, such as environmental and consumer products and in medical diagnostics.

Notes and references

Abbreviations

LoD = limit of detection (3 SD above the negative control); LoQ = limit of quantification (3 SD above the negative control); *E. coli = Escherichia coli (477414);* VTEC = verotoxin-producing *E. coli; A. acidoterrestris = Alicyclobacillus acidoterrestris*, recovery = the percentage of purified, inserted cell-equivalents; purification detection limit = the lowest concentration that can be purified, as determined by PCR; DNA yield = recovered DNA copies, rt-cycler = real-time cycler.

Associated content

The electronic supporting information (SI) contains more detailed materials and methods, details on the optical and heating design and supporting results.

Author contributions

The manuscript was written by MH; AHS and JS. MH; JS and AHS designed and interpreted experiments. MH and ESB performed all experiments. MH and ESB incorporated temperature-control and automated data analysis into the LabReader (invented by MH) and integrated a PCR tube in the LabTube platform. NP, AK, FvS and RZ invented and provided the CAD realization of the LabTube platform.

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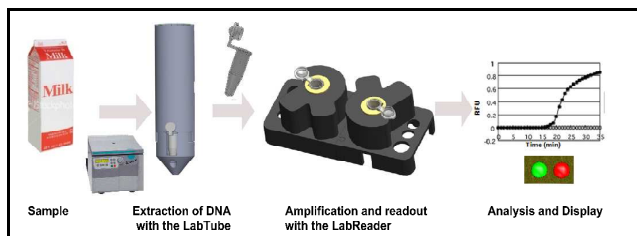
ToC Figure

The LabSystem for integrated, automated DNA purification, amplification and detection consists of a disposable, centrifugally-driven DNA purification platform (LabTube) and the subsequent amplification and detection in a low-cost UV/vis-reader (LabReader).