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A self-heating cartridge for molecular diagnostics†

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A disposable, water-activated, self-heating, easy-to-use, polymeric cartridge for isothermal nucleic acid amplification and visual fluorescent detection of the amplification products is described. The device is self-contained and does not require any special instruments to operate. The cartridge integrates chemical, water-triggered, exothermic heating with temperature regulation facilitated with a phase-change material (PCM) and isothermal nucleic acid amplification. The water flows into the exothermic reactor by wicking through a porous paper. The porous paper's characteristics control the rate of water supply, which in turn controls the rate of exothermic reaction. The PCM material enables the cartridge to maintain a desired temperature independent of ambient temperatures in the range between 20 °C and 40 °C. The utility of the cartridge is demonstrated by amplifying and detecting *Escherichia coli* DNA with loop mediated isothermal amplification (LAMP). The device can detect consistently as few as 10 target molecules in the sample. With proper modifications, the cartridge also can work with other isothermal nucleic acid amplification technologies for detecting nucleic acids associated with various pathogens borne in blood, saliva, urine, and other body fluids as well as in water and food. The device is suitable for use at home, in the field, and in poor-resource settings, where access to sophisticated laboratories is impractical, unaffordable, or nonexistent.

Introduction

In recent years, there has been a growing interest in inexpensive, point of care (POC) diagnostics for home care, individualized medicine, and therapeutics.[1–4] Such devices also address medical needs in regions of the world lacking sophisticated laboratory facilities. The lateral flow, immunochromatographic strip (often implemented in a 'dipstick' format) is perhaps the most successful example of a non-instrumented, point of care testing technology. Lateral flow (LF) strips are used, among other things, to detect antibodies to various infections such as the human immunodeficiency virus (HIV)[5,6] and malaria,[7] and to identify drugs of abuse[8] and test for pregnancy.[9] Although lateral flow immunoassay technology provides simple and rapid detection, it suffers from relatively low sensitivity and specificity. In many instances, it is desirable to detect low abundance analytes at concentrations well below the capabilities of the lateral flow assays. Additionally, in some cases, such as prior to HIV seroconversion[10–13] and during HIV therapy, antibodies are inadequate biomarkers.

Nucleic acids have the advantage that they can be amplified with great specificity, allowing the detection of just a few target molecules. The most frequently used nucleic acid amplification procedure is the polymerase chain reaction (PCR).[14–16] However, conventional PCR amplification requires elaborate sample preparation and complex thermal cycling. Since in most cases PCR requires costly processors, typically PCR is confined to centralized laboratories. There are just a few examples of portable, point of care, fully integrated PCR devices.[17,18] As an alternative to PCR, in recent years, various isothermal nucleic-acid amplification methods have been proposed.[19–22] Isothermal nucleic-acid amplification technologies are more suitable for low-cost, point of care (POC) diagnostics as they require less complex thermal control and consume less power than PCR, while often providing comparable sensitivity.[23]

Recent developments in microfluidic technology facilitate automated sample processing and miniaturization.[24–26] A variety of isothermal, enzymatic amplification-based microfluidic chips have been reported for POC testing applications. For example, Lutz *et al.*[27] demonstrated a recombinase polymerase amplification (RPA)-based lab-on-a-foil for detection of the antibiotic resistance gene *mecA* of *Staphylococcus aureus*. Gulliksen *et al.*[28] reported on a nucleic acid sequence-based amplification (NASBA) microfluidic chip for the detection of human papilloma virus (HPV). Sato *et al.*[29] demonstrated a microbead-based rolling circle amplification (RCA) microchip for *Salmonella* detection. Ramalingam *et al.*[30] combined helicase-dependent amplification (HDA) with microchip technology for amplifying the BNI-1 fragment of SARS cDNA. Fang *et al.*[31] reported a microchip-based, loop-mediated isothermal amplification

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(LAMP) system for pseudorabies virus (PRV) detection. Liu *et al.*[32,33] developed a single-chamber, microfluidic-based, LAMP cassette with an integrated isolation membrane for HIV detection in oral fluids and an integrated LAMP cassette with thermally actuated valves for *E. coli* DNA detection. The integration of the isolation membrane within the reaction chamber allows for target isolation, concentration, and removal of inhibitors while at the same time simplifying flow control.

In all the cases reported above, temperature control was provided with electrical power and required electronic temperature regulation, which increases cost. To eliminate the need for electrical power and electronic circuits, a number of researchers have proposed exothermic chemical reaction-based heating. The idea of heat production with exothermic reactions is not new. Chemical reactions have been used for, among other things, heating coffee and soup cans, pads for trauma patients, outdoor hand-warmers, and for sterilizing medical equipment and waste.[34–37] More recently, Weigl *et al.*[38,39] proposed non-instrumented and/or minimally instrumented diagnostic devices and mentioned, without any details, a non-instrumented LAMP device heated with an exothermic reaction. LaBarre *et al.*[40] reported on a non-instrumented nucleic acid amplification (NINA) device for malaria molecular diagnostics, in which the PCR vials are embedded in a calcium-oxide heat source that is thermally coupled to a phase change material for temperature regulation. Hatano *et al.*[41] have successfully carried out LAMP amplification of *Bacillus anthracis* pag and capB gene fragments in PCR vials embedded in commercially available heating packs of the type used for hand warmers. However, none of the above reports addresses the integration of the reaction chamber with microfluidics.

In this article, we describe, for the first time, a novel, disposable, water-activated, self-heating, microfluidic cartridge for isothermal nucleic acid amplification. The device does not require any external instrument and/or power. The device is powered by an exothermic chemical reaction ('thermal battery'). The heat is generated by reacting magnesium with water in the presence of iron. The water wicks into a chamber containing the magnesium–iron powder through a filter paper, which allows one to control the water flow rate and thus the exothermic reaction rate. The reactor's temperature is regulated and rendered independent of ambient temperatures with the aid of a phase change material (PCM). The utility of this integrated, self-heating cartridge is demonstrated by amplifying *E. coli* DNA with the LAMP process and visually detecting the amplification products. The cartridge is particularly suitable for use in the field, in resource-poor regions of the world (where funds and trained personnel are in short supply), in remote areas without electricity, and at home.

Water-activated, self-heating cartridge

An exploded view of the water-activated, self-heating, non-instrumented cartridge for isothermal nucleic acid amplification is shown in Fig. 1. Fig. 2 features a photograph of the device. The 38 mm length × 19 mm width × 17 mm height, polymethyl methacrylate (PMMA) cartridge consists of two main components: a cartridge body and a cartridge seat. Both components are made with 5.5 mm (0.216 inch) thick, PMMA sheets.

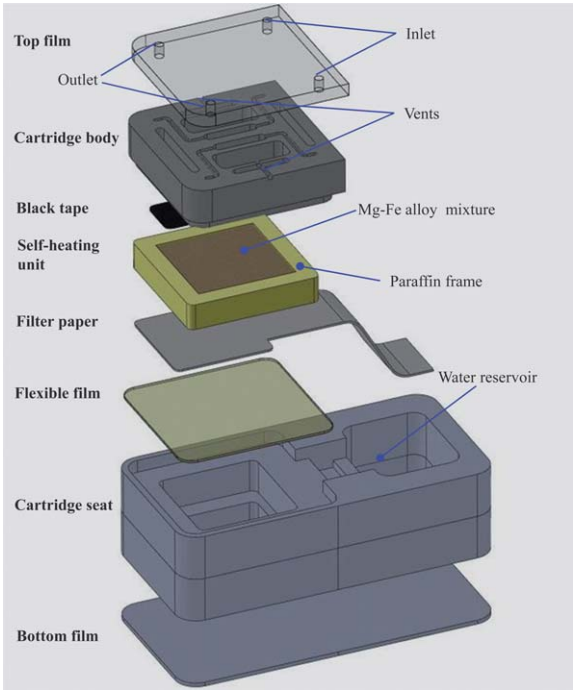


Fig. 1 Exploded view of the water-activated, self-heating, non-instrumented cartridge for isothermal amplification of nucleic acids. The cartridge consists of two main components: a cartridge body and a cartridge seat.

The cartridge and its components are milled with a precision, computer numerical control (CNC) milling machine (HAAS Automation Inc., Oxnard, CA, USA). The component PMMA layers are solvent-bonded with acetonitrile (Sigma-Aldrich) at room temperature to form a laminated microfluidic cartridge. Residual solvent was removed by overnight heating at 50 °C.

The cartridge body contains two amplification chambers (a greater number of amplification chambers can be accommodated, when desired) and a self-regulated, exothermic reaction chamber. Each amplification chamber is 6.3 mm in length, 1.3 mm in width, 1.9 mm in depth, and 15.6 μl in volume. The amplification chambers are located at the top of the self-heating

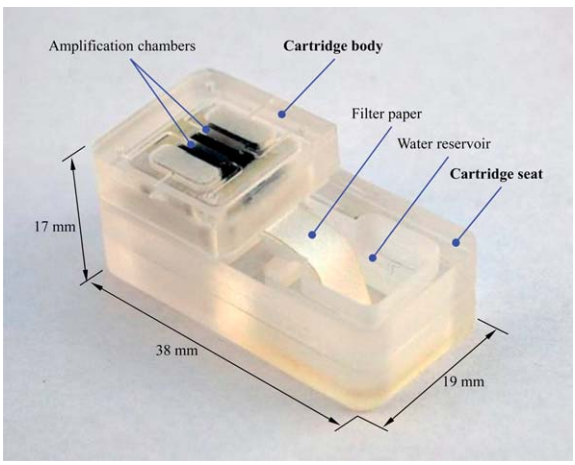


Fig. 2 A photograph of the water-activated, self-heating, non-instrumented cartridge for isothermal amplification of nucleic acids.

chamber. The location of the amplification chambers allows for easy optical access to the amplification chambers. The cartridge body is capped with a 1.5 mm (0.06 inch) thick, transparent PMMA sheet. Inlet and outlet ports to the amplification chambers are formed as vials in the cap film. The vials are connected to the amplification chambers through conduits with a cross-section of 500 μm × 500 μm milled in the upper surface of the cartridge body.

The self-heating chamber (thermal battery) is 16 mm in length, 16 mm in width, and 3.2 mm in depth. The heating chamber contains Mg–Fe alloy[42,43] (MRE Info. USA, see Fig. S1 of ESI†) surrounded with a custom-cast, paraffin (Sigma-Aldrich) frame (see Fig. S2A of ESI†). The magnesium reacts with water ($Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2(g)$) to produce heat. The gases produced during the reaction are discharged to the atmosphere through vents fabricated in the cartridge body. The melting temperature of the paraffin is 65 °C. The paraffin regulates the reaction chamber's temperature and, due to its low conductivity, also insulates the exothermic reaction chamber. To improve thermal isolation, both the amplification chambers and the self-heating chamber are surrounded with air-filled cavities. A piece of carbon black, double-sided, adhesive tape (Nisshin EM Corporation, Tokyo) is placed underneath the amplification chamber to reduce background fluorescence emission. The cartridge seat also houses a water reservoir and is capped at the bottom with a 250 mm (0.01 inch) thick PMMA film. The water reservoir has a volume of 1.5 ml and is connected to the self-heating chamber with a strip of filter paper.

The 16 mm length × 16 mm width × 3.2 mm height paraffin frame was cast in a PDMS mold placed on a hot plate at 80 °C. The fabrication of the paraffin frame is described in Fig. S2B (ESI†). The frame was then inserted into the self-heating chamber and filled with 0.36 g of Mg–Fe alloy mixture. The mass of the alloy was adjusted to meet the needs of the device. Greater mass of alloy could maintain the device at the desired temperature for a longer period of time.

The paper strip was cut from a Whatman No. 1 filter paper (7 cm diameter, Whatman, Hillsboro, OR) with a CO_2 laser machine (Universal Laser Systems, Scottsdale, AZ). The filter paper strip provided a hydraulic connection between the water reservoir and the exothermic reaction chamber. A piece of 19 mm length × 19 mm width plastic food wrap (Polyvinyl Films, Inc., Sutton, MA) is positioned beneath the porous strip to prevent any powdered reagents from spilling out of the reaction chamber. Prior to the start of heating, either the water reservoir was filled with water or the filter paper was brought into contact with pre-stored water in the water reservoir. During operation, the reaction's chamber temperature remained under 80 °C. Visual inspection of the paper strip after one hour operation did not reveal any damage.

Temperature measurement and calibration

To evaluate the thermal performance of the reaction chambers, we constructed a calibration cartridge. The calibration cartridge was identical to the actual cartridge with the addition of a 1.0 mm diameter hole drilled in the top of the reaction chamber. A K-type thermocouple (Omega Engineering, 75 μm in diameter wires, and a junction diameter of ~170 μm) was inserted in the reaction chamber through the drilled hole and sealed in place. The thermocouple wires were connected to a terminal block (SCC-68) that interfaced with a National Instruments data acquisition card (PXI-6281). The reaction chamber was then filled with DI water and its vials were sealed by using transparent tape (Scotch tape, 3 M, St Paul, MN) to prevent liquid evaporation during the heating process. Once the chemical, exothermic reaction was initiated by adding water into its water reservoir, the temperature was monitored and displayed using Labview™ software (National Instruments, Austin, TX, USA).

To examine the effect of ambient temperature on the amplification chamber's temperature, experiments were carried out both at room temperature and at elevated temperatures (30 °C and 40 °C) in an oven (Isotemp Vacuum Oven Model 280A, Fisher Scientific Inc., Pittsburgh, PA). The setup for the calibration experiments is shown in Fig. S3 of ESI†.

E. coli DNA LAMP protocol

To demonstrate the applicability of the self-heating cartridge, we used the cartridge to amplify *E. coli* DNA. The LAMP template (plasmid DNA) extracted from pathogenic *E. coli* in urine samples and the primers for the *E. coli* amplification were a gift from Professor Abhay Vats, MD (Department of Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania). The primers and their respective concentrations for *E. coli* DNA amplification were: outer primer F3 5′-GCCATCTCCTGA TGACGC-3′ (0.2 μM), outer primer B3 5′-ATTTACCG CAGCCAGACG-3′ (0.2 μM), loop primer F loop 5′-CTTTGTAACAACCTGTCATCGACA-3′ (0.8 μM), loop primer B loop 5′-ATCAATCTCGATATCCATGAAGGTG-3′ (0.8 μM), inner primer BIP 5′-CTGGGGCGAGGTCGTGG TATTCCGACAAACACCACGAATT-3′ (1.6 μM), and inner primer FIP 5′-CATTTTGCAGCTGTACGCTCGCAGCC CATCATGAATGTTGCT-3′ (1.6 μM).[44] The reaction mix also contained 20 mM Tris–HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_2)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100 (Sigma Chemical Co., MO), 0.8 M betaine (Sigma-Aldrich, St Louis, MO), 8 units of Bst DNA polymerase (New England Biolabs, Inc., MA), 1.4 mM dNTPs, 15.0 μM SYTO® 9 Green (Molecular Probes, Inc., Eugene, OR), and 5 μl of *E. coli* DNA at various concentrations. DNase/RNase-free water was used throughout the LAMP experiments.

Cartridge operation and visual fluorescent detection

First, 20 μl of LAMP master mix, which contains all the reagents necessary for LAMP, the fluorescent dye (SYTO® 9 Green), and the target molecules were injected into one of the amplification chambers through its inlet port. A similar mixture without the target DNA was injected into the second amplification chamber, which was used as a control. Next, the inlet and outlet ports were sealed using transparent tape to minimize evaporation and contamination during the amplification process. Then, 800 μl of tap water was added into the water reservoir of the cartridge with a pipette (Movie S1, ESI†). The water wicked slowly through the filter paper strip into the exothermic reaction chamber to initiate and sustain the exothermic chemical reaction.

After about 1 hour, the cartridge was illuminated with a reusable, keychain-mounted UV light (UV money detector, PickEgg Ltd, Hong Kong, ~$2 unit price, shown in Fig. S4 of ESI† (ref. 45)) in a dark room. The green fluorescence emission from the two reaction chambers was directly observed by eye and/or recorded with a portable digital camera (Sony, DCR-PC330, Japan).

To confirm the visual fluorescence end-point detection results, the LAMP reaction products were recovered from the amplification chambers with a pipette and analyzed by gel electrophoresis. 5 μl of each LAMP-amplified product was loaded onto a lane of a 2.0% agarose gel. Electrophoresis of the amplified DNA fragment was carried out in TAE (Tris–acetate–EDTA) buffer at a constant potential difference of 114 V for 40 minutes. DNA marker VIII (Roche Diagnostic, Indianapolis, Indiana, USA) was concurrently used to gauge the sizes of the amplified DNA bands. The gel was stained with 1% ethidium bromide and was visualized with UV illumination.

Results and discussion

Flameless exothermic heater

We selected the commercially available, flameless ration heater (FRH) as our cartridge's self-heating material due to its ease of use, biodegradability, and safety. FRH is a water-activated, exothermic chemical heater that is included with Meals, Ready-to-Eat (MREs).[42,43] This FRH consists of a powder mixture of Mg–Fe alloy, NaCl, antifoaming agents, and inert filler (Fig. S1 in the ESI†).

In the presence of excess water, the exothermic chemical reaction between the Mg–Fe alloy and the water occurs rapidly. The temperature can increase to well above 90 °C in several seconds. To control the rate of the reaction, we control the rate of the water supply into the reaction chamber. We used a porous, hydrophilic, filter paper strip to bridge the water reservoir and the exothermic reaction chamber. The filter paper was selected because of its good wetability, porous structure, and mechanical strength. Once water is added into the water reservoir, it is absorbed by the filter paper and steadily wicks into the exothermic reaction chamber by capillary action without a need for any external pumps. The rate of water flow depends on the width of the strip.

To evaluate the effect of the filter paper strip's size on the amplification chamber's temperature, three filter paper strips with different widths (5.0 mm, 7.0 mm and 9.0 mm) were tested at room temperature (~22 °C) and in the absence of the paraffin frame. Fig. 3 depicts the exothermic reaction chamber's temperature as a function of the strips' width. The wider the filter paper strip, the higher the amplification chamber's temperature. This experiment illustrates that the amplification chamber's temperature can be controlled by adjusting the width of the filter paper strip. In all the experiments described later in the paper, we used a 7.0 mm wide filter paper strip as it enabled amplification chamber temperatures between 60 and 65 °C, which is the desired temperature range for the LAMP reaction.

Temperature-regulation with the phase change material

In the previous section, we have demonstrated that when the ambient temperature is fixed, the amplification chamber's temperature can be controlled by adjusting the rate of the water supply. For the device to be useful outdoors, it is necessary to decouple the amplification chamber's temperature from ambient conditions. We accomplish this objective with the use of the phase change material. To demonstrate the regulating effect of the phase change material, we monitored the amplification chamber's temperature in the absence and presence of the phase change material at three different ambient temperatures: 22 °C, 30 °C, and 40 °C. The elevated ambient temperatures were maintained by carrying out the experiment in an oven (Fig. S3, ESI†). Fig. 4 depicts the average reaction chamber temperature in the absence (dark, blue bars) and presence (light, gray bars) of the phase change material as a function of the ambient temperature. The reported temperature is the amplification chamber's average temperature during a one hour period (once the chamber's temperature was ramped up from its ambient value to the desired amplification temperature). The error bars correspond to the scatter of the temperature in three devices. In the absence of the moderating effect of the phase change material, the amplification chamber's temperature increased from 63 °C at the ambient temperature of 22 °C to 75 °C at the ambient

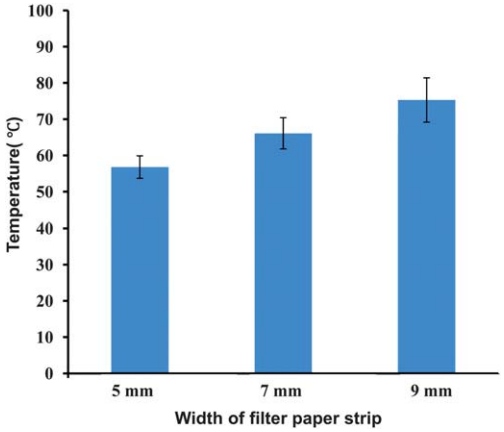


Fig. 3 The amplification chamber's temperature as a function of the filter paper strip's width (n = 3). The room temperature is ~22 °C (without paraffin frame).

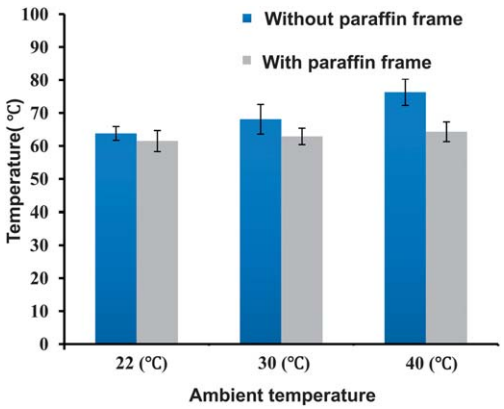


Fig. 4 The amplification chamber's temperature as a function of the ambient temperature in the absence (dark, blue bars) and presence (light, gray bars) of a regulating phase change material (n = 3).

temperature of 40 °C. Although the LAMP process is quite forgiving to temperature variations, temperatures above 70 °C are likely to adversely affect the activity of the polymerase enzymes. In contrast, in the presence of the phase change material, the amplification chamber's temperature was nearly independent of the ambient temperature and was retained at 64 ± 3 °C even when the ambient temperature was 40 °C. The figure demonstrates that the paraffin can successfully regulate the temperature by absorbing excess heat as latent heat during melting and, if necessary, releasing the stored heat during solidification.

Fig. 5 shows the amplification chamber's temperature as a function of time when the ambient temperatures are 22 °C (black, solid line), 30 °C (blue, dashed line), and 40 °C (red, dotted line). Time zero is set to coincide with the instant when water is added into the water reservoir. Once water has been introduced into the cartridge, it takes nearly 10 minutes for the water to migrate into the exothermic reaction chamber and to raise the cartridge's temperature to ~60 °C. In this experiment, we used 0.36 g of Mg–Fe alloy mixture and 0.8 ml of water. The amount of the reactants used in our experiments is about 50 fold smaller than previously reported in a self-heating experiment.[40] When the ambient temperature is 22 °C, the amplification chamber's temperature ranged from 59.3 to 62.5 °C for about an hour, which is longer than typically necessary to amplify the target molecules to detectable levels. Since the LAMP reaction operates effectively over a relatively broad temperature range[41] (*i.e.*, 60–65 °C), the temperature range maintained in our experiments is quite adequate. It is likely that, with appropriate optimization and the use of multiple exothermic reaction chambers, the time needed to ramp up the temperature can be considerably shortened.

E. coli DNA amplification and visual detection

To demonstrate that the device can be used to carry out the amplification process, we amplified a target DNA from the bacterium *E. coli*. *E. coli* is the second major cause of neonatal meningitis, which is a significant contributor of mortality among newborns.[46–50] It is also associated with a high incidence of neurological sequelae.

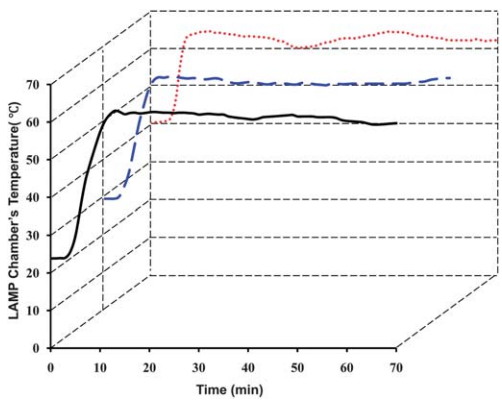
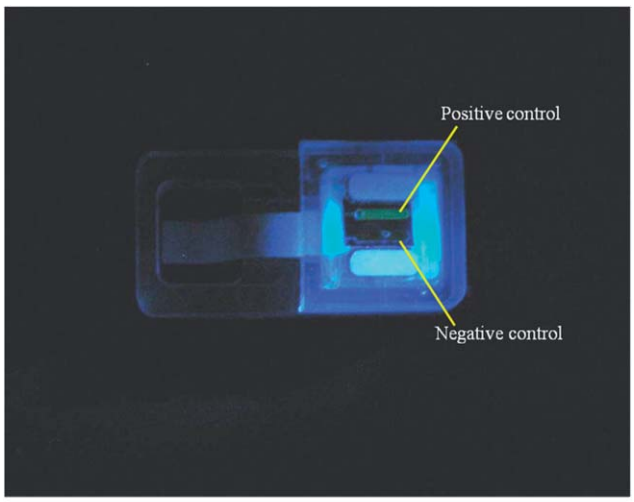
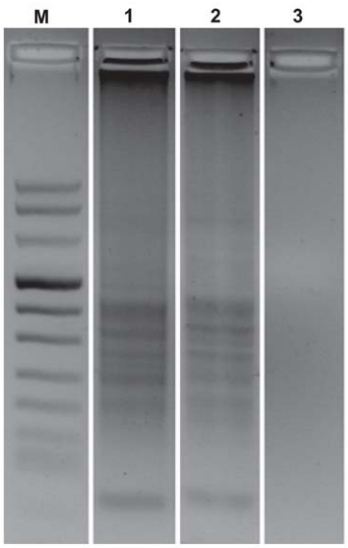


Fig. 5 The amplification chamber's temperature as a function of time when the ambient temperatures are 22 °C (black solid line), 30 °C (blue dashed line), and 40 °C (red dotted line).

Since the LAMP process is highly efficient, it is capable of amplifying low abundance targets to detectable quantities within less than an hour. LAMP products can be directly detected by visual observation of end-point turbidity or fluorescence with the naked eye.[31,51] Here, we use the fluorescent emission of the intercalating dye SYTO® Green to directly observe the LAMP products. To avoid interference from background fluorescence emitted by the PMMA cartridge itself and the Mg–Fe alloy, we inserted a piece of carbon black, double-sided adhesive tape underneath the amplification chambers. In separate experiments (not reported here), we determined that the carbon black tape emits negligible background fluorescence.[52]



(A)



(B)

Fig. 6 (A) Green fluorescence emission from a test amplification chamber in which we amplified ten target *E. coli* DNA molecules and from a negative control reaction chamber. The excitation source is a mini keychain UV light-emitting diode. (B) Electropherograms of LAMP products (5.0 μl) of *E. coli* DNA in a 2% agarose gel. Lane M, DNA marker VIII. Lanes 1, 2, and 3 correspond, respectively, to 100, 10, and 0 (negative control) target copies per reaction chamber.

Fig. 6A shows the fluorescence images of the *E. coli* positive sample (10 target copies) and negative sample (0 target molecules, *i.e.*, no template) in the self-heating LAMP cartridge after LAMP amplification at room temperature (~22 °C) for 60 minutes. The reaction chambers were excited with a low cost (~$2.00), reusable UV source (which is commonly used to detect counterfeit currency). There is a distinct difference between the signal intensity emitted by the test chamber compared with that emitted by the negative control chamber. We also observe a non-negligible amount of emission from the paraffin frame. The emission from the paraffin frame can be eliminated by encircling the reaction chambers with black tape.

The use of *in situ* fluorescent detection simplifies cartridge design and operation as it eliminates the need to transfer the reaction products from the amplification chamber to the detection chamber. The *in situ* fluorescence detection also reduces the analysis time. The amplification chamber can be illuminated at various stages of the process and the test can be concluded as soon as a detectable signal is observed. The disposable device described here does not require any instrumentation or external power to operate.

Variants of the device can include continuous detection of the fluorescent emission with a CCD camera instead of the end-point detection described herein. The use of a camera would allow measurement of emitted signal intensity as a function of time and quantification. Yet another variant of the device can include discharge of the amplification products (when appropriately functionalized primers are used) onto a lateral flow strip. The lateral flow strip can offer the ability of detecting multiple targets and provides a more permanent record of the test.

To verify the visual detection results, we subjected the LAMP products to gel electrophoresis. Fig. 6B shows a sample of electropherograms of ethidium bromide, stained LAMP products in 2% agarose gel. Lane M is the DNA marker VIII. Lanes 1, 2, and 3 correspond, respectively, to LAMP cartridge products with 100, 10, and 0 (negative control) copies. The results clearly indicate that as few as 10 *E. coli* DNA copies can be comfortably detected on our cartridge. Each experiment was repeated three times with similar results. This is a comparable performance to that of state of the art benchtop thermocyclers as well as with our integrated cartridge which was heated with a thin film heater.[32]

Conclusions and outlook

This work is the first report of a low-cost, disposable, water-activated, self-heating, non-instrumented, microfluidic cartridge for nucleic acid amplification and detection. The device utilizes the exothermic reaction between Mg–Fe alloy and water as the heat source. The reaction rate is controlled by using a filter paper to control the water flow rate from a water reservoir to the exothermic reaction chamber. The amplification chambers' temperatures are regulated with a phase change material (*i.e.*, paraffin) and maintained at a desired level, suitable for LAMP-based amplification, independent of the ambient temperature, over a wide range of ambient temperatures ranging from 20 °C to 40 °C.

To demonstrate the device's utility for nucleic acid amplification, we amplified and detected *E. coli* DNA and were able to comfortably detect down to 10 target molecules in the sample. Although it is quite possible that the device can provide a better detection limit, perhaps down to a single molecule, substantiating such a claim would require a large number of experiments to obtain statistically meaningful data. Since currently our devices are hand produced with a limited budget, we are not in a position to test dozens of cartridges. The performance of the device appears to be comparable to that of the state of the art, benchtop PCR machines and with our integrated cartridge heated with a thin film heater.[33]

We monitored the amplification results by exciting a fluorescent signal with a mini, reusable, inexpensive (<$2) keychain UV light-emitting diode (LED) and observed the emitted signal by eye without any instrumentation. The visual test can provide only qualitative results. By monitoring the emission with a CCD camera, such as is available in cell phones, or photodiodes, one should be able to measure signal intensity as a function of time and quantify the number of target molecules in the sample.

If desired, the device can be interfaced with a lateral flow strip and the amplification products can be detected with labels such as gold particles. Lateral flow strip based detection would require, however, the discharge of the products onto a lateral flow strip, which would require additional flow control. The lateral flow strip may have, however, the advantage of allowing for the concurrent identification of multiple targets when a multiplexed LAMP reactor is used.

With the addition of sample preprocessing of the type that we described in an earlier work,[32] our preliminary experiments indicate that the self-heated cartridge system has the potential for rapid, inexpensive diagnosis of infectious diseases at the point of care. LAMP is, of course, just one example of an isothermal amplification process. With proper modification, the cartridge also can work with other isothermal nucleic acids amplification techniques.

Future modifications and improvements of the cartridge will include dry storage of LAMP/RT-LAMP reagents in the amplification chamber. This can be achieved by encapsulating the dry reagents with paraffin,[53] which will melt upon heating of the reaction chamber to the desired incubation temperature, move out of the way, and allow the hydration of the reagents. Another improvement may include equipping the cartridge with a solid phase membrane for the isolation, concentration, and purification of nucleic acid targets.[32]

The fully integrated, non-instrumented, nucleic acid testing cartridge can be operated at the point of care by minimally trained personnel and can carry out all the necessary steps from sample to answer. With appropriate modifications of the reagents, the system can be used to detect various infectious diseases, monitor the health of individuals, provide a trigger for the administration of expensive or dangerous medications, and facilitate monitoring water and food quality. The device is suitable for use in the field, in resource-poor regions, especially in areas without reliable electric power, in remote areas, and at home.

Acknowledgements

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References

1 P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam and B. H. Weigl, *Nature*, 2006, **442**, 412–418.
2 X. Cheng, A. Gupta, C. Chen, R. Tompkins, W. Rodriguez and M. Toner, *Lab Chip*, 2009, **9**, 1357–1364.
3 J. V. Jokerst, P. N. Floriano, N. Christodoulides, G. W. Simmons and J. T. McDevitt, *Lab Chip*, 2008, **8**, 2079–2090.
4 K. A. Curtis, D. L. Rudolph and S. M. Owen, *J. Virol. Methods*, 2008, **151**, 264–270.
5 C. Liu, X. Qiu, S. Ongagna, D. Chen, Z. Chen, W. R. Abrams, D. Malamud, P. L. A. M. Corstjens and H. H. Bau, *Lab Chip*, 2009, **9**, 768–776.
6 X. Qiu, J. A. Thompson, Z. Chen, C. Liu, D. Chen, S. Ramprasad, M. Mauk, S. Ongagna, C. Barber, W. Abrams, D. Malamud, P. Corstjens and H. Bau, *Biomed. Microdevices*, 2009, **11**, 1175–1186.
7 C. K. Murray, R. A. Gasser, A. J. Magill and R. S. Miller, *Clin. Microbiol. Rev.*, 2008, **21**, 97–110.
8 R. Wong, *Am. Clin. Lab*, 2002, **21**, 37–39.
9 Inverness Medical Innovations Inc, Clearblue Pregnancy Tests, http://www.alere.com/en/global-products-services.html.
10 M. A. Dineva, L. MahiLum-Tapay and H. Lee, *Analyst*, 2007, **132**, 1193–1199.
11 F. Rouet, D. K. Ekouevi, M. L. Chaix, M. Burgard, A. Inwoley, T. D. Tony, C. Danel, X. Anglaret, V. Leroy, P. Msellati, F. Dabis and C. Rouzioux, *J. Clin. Microbiol.*, 2005, **43**, 2709–2717.
12 N. Mehta, S. Trzmielina, B. A. Nonyane, M. N. Eliot, R. Lin, A. S. Foulkes, K. McNeal, A. Ammann, V. Eulalievyolo, J. L. Sullivan, K. Luzuriaga and M. Somasundaran, *PLoS One*, 2009, **4**, e5819.
13 D. Gibellini, F. Vitone, E. Gori, M. L. Placa and M. C. Re, *J. Virol. Methods*, 2004, **115**, 183–189.
14 C. Zhang and D. Xing, *Nucleic Acids Res.*, 2007, **35**, 4223–4237.
15 J. Wang, Z. Chen, P. L. A. M. Corstjens, M. G. Mauk and H. H. Bau, *Lab Chip*, 2006, **6**, 46–53.
16 S. H. Lee, S. W. Kim, J. Y. Kang and C. H. Ahn, *Lab Chip*, 2008, **8**, 2121–2127.
17 Z. Chen, M. G. Mauk, J. Wang, W. R. Abrams, P. L. A. M. Corstjens, R. S. Niedbala, D. Malamud and H. H. Bau, *Ann. N. Y. Acad. Sci.*, 2007, **1098**, 429–436.
18 D. Chen, M. G. Mauk, X. Qiu, C. Liu, J. Kim, S. Ramprasad, S. Ongagna, W. R. Abrams, D. Malamud, P. L. A. M. Corstjens and H. H. Bau, *Biomed. Microdevices*, 2010, **12**, 705–719.
19 J. Compton, *Nature*, 1991, **350**, 91–92.
20 G. T. Walker, M. S. Fraiser, J. L. Schram, M. C. Little, J. G. Nadeau and D. P. Malinowski, *Nucleic Acids Res.*, 1992, **20**, 1691–1696.
21 O. Piepenburg, C. H. Williams, D. L. Stemple and N. A. Armes, *PLoS Biol.*, 2006, **4**, e204.
22 T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino and T. Hase, *Nucleic Acids Res.*, 2000, **28**, E63.
23 P. J. Asiello and A. J. Baeumner, *Lab Chip*, 2011, **11**, 1420–1430.
24 P. Liu, X. Li, S. A. Greenspoon, J. R. Scherer and R. A. Mathies, *Lab Chip*, 2011, **11**, 1041–1048.
25 X. Chen, D. Cui, C. Liu, H. Li and J. Chen, *Anal. Chim. Acta*, 2007, **584**, 237–243.
26 C. Liu, *Microfluid. Nanofluid.*, 2010, **9**, 923–931.
27 S. Lutz, P. Weber, M. Focke, B. Faltin, J. Hoffmann, C. Müller, D. Mark, G. Roth, P. Munday, N. Armes, O. Piepenburg, R. Zengerle and F. von Stetten, *Lab Chip*, 2010, **10**, 887–893.
28 A. Gulliksen, L. A. Solli, K. S. Drese, O. Sörensen, F. Karlsen, H. Rogne, E. Hovig and R. Sirevåg, *Lab Chip*, 2005, **5**, 416–420.
29 K. Sato, A. Tachihara, B. Renberg, K. Mawatari, K. Sato, Y. Tanaka, J. Jarvius, M. Nilsson and T. Kitamori, *Lab Chip*, 2010, **10**, 1262–1266.
30 N. Ramalingam, T. C. San, T. J. Kai, M. Y. M. Mak and H. Q. Gong, *Microfluid. Nanofluid.*, 2009, **7**, 325–336.
31 X. Fang, Y. Liu, J. Kong and X. Jiang, *Anal. Chem.*, 2010, **82**, 3002–3006.
32 C. Liu, E. Geva, M. Mauk, X. Qiu, W. R. Abrams, D. Malamud, K. Curtis, M. Owen and H. H. Bau, *Analyst*, 2011, **136**, 2069–2076.
33 C. Liu, M. Mauk and H. H. Bau, *Microfluid. Nanofluid.*, 2011, DOI: 10.1007/s10404-011-0788-3.
34 D. L. Collins, *US pat.*, 6231596, May 15, 2001.
35 S. L. Feld, *US pat.*, 4106477, August 15, 1978.
36 M. W. Sabin and C. M. Sabin, *US pat.*, 5984953, November 16, 1999.
37 C. Sharma, R. K. Sharma, C. Kant and A. K. Sarkar, *US pat.*, 6537309, March 26, 2001.
38 B. Weigl, G. Domingo, P. Labarre and J. Gerlach, *Lab Chip*, 2008, **8**, 1999–2014.
39 B. Weigl, G. Domingo, J. Gerlach, D. Tang, D. Harvey, N. Talwar, A. Fichtenholz, B. van Lew and P. LaBarre, *Proc. SPIE*, 2008, **6886**, 688604.
40 P. LaBarre, J. Gerlach, J. Wilmoth, A. Beddoe, J. Singleton and B. Weigl, *Conf. Proc. IEEE Eng. Med. Biol. Soc.*, 2010, 1097–1099.
41 B. Hatano, T. Maki, T. Obara, H. Fukumoto, K. Hagisawa, Y. Matsushita, A. Okutani, B. Bazartseren, S. Inoue, T. Sata and H. Katano, *Jpn. J. Infect. Dis.*, 2010, **63**, 36–40.
42 M. Lamensdorf, *US pat.*, 5611329, August 04, 1995.
43 http://www.mreinfo.com/.
44 J. Hill, S. Beriwal, I. Chandra, V. K. Paul, A. Kapil, T. Singh, R. M. Wadowsky, V. Singh, A. Goyal, T. Jahnukainen, J. R. Johnson, P. I. Tarr and A. Vats, *J. Clin. Microbiol.*, 2008, **46**, 2800–2804.
45 Mini Keychain UV Light, http://www.pickegg.com/.
46 S. C. Clarke, *Diagn. Microbiol. Infect. Dis.*, 2001, **41**, 93–98.
47 S. Bonacorsi and E. Bingen, *Int. J. Med. Microbiol.*, 2005, **295**, 373–381.
48 J. R. Johnson, *Infect. Dis. Clin. North Am.*, 2003, **17**, 261–278.
49 E. J. Klein, D. R. Boster, J. R. Stapp, J. G. Wells, X. Qin, C. R. Clausen, D. L. Swerdlow, C. R. Braden and P. I. Tarr, *Clin. Infect. Dis.*, 2006, **43**, 807–813.
50 C. F. Marrs, L. Zhang and B. Foxman, *FEMS Microbiol. Lett.*, 2005, **252**, 183–190.
51 Y. Mori, K. Nagamine, N. Tomita and T. Notomi, *Biochem. Biophys. Res. Commun.*, 2001, **289**, 150–154.
52 C. Liu, J. A. Thompson and H. H. Bau, *Lab Chip*, 2011, **11**, 1688–1693.
53 J. Kim, D. Byun, M. G. Mauk and H. H. Bau, *Lab Chip*, 2009, **9**, 606–612.

