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Instrument-free exothermic heating with phase change temperature control for paper microfluidic devices

Jered Singleton¹, Chris Zentner¹, Josh Buser², Paul Yager², Paul LaBarre¹, and Bernhard H. Weigl¹

Jered Singleton: jsingleton@path.org; Chris Zentner: czentner@path.org; Josh Buser: buserj@uw.edu; Paul Yager: yagerp@uw.edu; Paul LaBarre: plabarre@path.org; Bernhard H. Weigl: bweigl@path.org

¹PATH, 2201 Westlake Ave., Suite 200, Seattle, WA, USA 98121

²Department of Bioengineering, University of Washington, Seattle, WA, USA 98195, Telephone: (206) 285-3500; Fax: (206) 285-6619

Abstract

Many infectious diseases, as well as some cancers, that affect global health are most accurately diagnosed through nucleic acid amplification and detection. There is a great need to simplify nucleic acid-based assay systems for use in global health in low-resource settings as well as in settings that do not have convenient access to laboratory staff and equipment such as doctors' offices and home care settings.

In developing countries, unreliable electric power, inadequate supply chains, and lack of maintenance for complex diagnostic instruments are all common infrastructure shortfalls. Many elements of instrument-free, disposable, nucleic acid amplification assays have been demonstrated in recent years. However, the problem of instrument-free,¹ low-cost, temperature-controlled chemical heating remains unsolved. In this paper we present the current status and results of work towards developing disposable, low-cost, temperature-controlled heaters designed to support isothermal nucleic acid amplification assays that are integrated with a two-dimensional paper network. Our approach utilizes the heat generated through exothermic chemical reactions and controls the heat through use of engineered phase change materials to enable sustained temperatures required for nucleic acid amplification. By selecting appropriate exothermic and phase change materials, temperatures can be controlled over a wide range, suitable for various isothermal amplification methods, and maintained for over an hour at an accuracy of $\pm 1^\circ\text{C}$.

Keywords

non-instrumented nucleic acid amplification-based assay platform; sample preparation; amplification; chemical heating; phase change material temperature stabilization; membrane-based amplicon detection

¹By instrument-free diagnostics we refer to assays that are self-contained, disposable, storable, and generally easy to use by minimally trained operators or lay people. They do not need electronic readers or any other significant laboratory equipment or supplies to be used.

1. Introduction

Some frustrations with the complexities of most traditional plastic- or glass-based microfluidic diagnostic disposables have led to the development of devices that make use of capillary action to drive fluids through networked paper channels. These methods offer promise for creating devices that are robust, low-cost, and reliable. A continually increasing number of functions are currently being designed into two-dimensional paper networks, which will allow for greater diagnostic device functionality in the future.¹⁻⁷ To take advantage of the single-use, disposable nature of paper microfluidic diagnostic devices, a compatible, equally low-cost, disposable heater is required.

Over the past decade, funders, industry, and other groups have demonstrated unprecedented interest in developing novel diagnostic technologies that specifically address the needs of low-resource settings. Much of this interest has focused on point-of-care (POC) diagnostic testing.⁸

One possible manifestation of POC diagnostic testing involves the use of portable instrumented tests capable of being transported between clinics, laboratories, and other health care settings. These devices, however convenient for patients and providers, still require the initial capital investment of the instrument as well as the associated costs of repairing and maintaining the device. In order to offset these expenses, a minimal number of tests must be run on the instrument over its life. For this reason, procurement decisions for equipment are based on a sufficient patient volume along with insurance reimbursement programs. In developed countries, insurance reimbursement allows hospitals to offset the initial cost along with maintenance and repair costs. This is not the case in developing countries or in rural settings in developed countries where patient volume may be low and patients are without adequate insurance. In these settings and others, non-instrumented POC tests would, therefore, be of great benefit. These tests would also open up unexplored markets for use by patients at home and for field use by the military and emergency response teams.⁹

Disposable assays requiring no instrument, such as immunochromatographic strip tests, have been very successful in low-resource settings, with hundreds of millions of such rapid diagnostic tests sold and used annually in developing countries. However, such assays generally work on the immunoassay principle and cannot be used to directly detect nucleic acids in low copy numbers from pathogens or cancer cells. There is a growing body of literature emphasizing the need for medical diagnostics that can combine the simplicity of such lateral flow tests with the sensitivity and specificity of a polymerized chain reaction (PCR) test.¹⁰⁻¹² Many process elements of non-instrumented, disposable nucleic acid amplification tests (NAATs) have been demonstrated in recent years.²

A number of diagnostic processes used in these NAATs and other diagnostic devices require heat and temperature control to allow for proper device function, including the following:

1. Nucleic acid amplification: Many isothermal NAAT processes are now available that need to be maintained at a single temperature (typically between 35°C and 70°C) for a period of time ranging from minutes to an hour.^{13,14} Such heaters need

to maintain an exact temperature (± 1 or 2°C) in a range of ambient temperatures for optimal enzyme performance, since amplification is enzymatic.

2. Lysis: Some pathogens and other cellular materials are especially difficult to lyse with chemical lysis alone, and chemical lysis can also interfere with downstream processes including amplification. Heat lysis is an interesting alternative that has been demonstrated for tuberculosis and other pathogens.^{15,16}
3. Drying: Sometimes drying of a biological material or other sample is performed as part of an analytical process; drying can facilitate lysis or concentration of the analyte. Drying is enhanced by elevated temperatures.
4. Incubation: Live biologic materials, especially pathogen cultures as well as enzymatic processes, sometimes need to be incubated to an elevated temperature (frequently 37°C) to properly grow or function.
5. Analytical reaction temperature control: Almost all reactions have a temperature dependence that can influence their analytic performance. Controlling the temperature can enhance precision. In some cases the assay is only functional within a tight range of temperatures.

If non-instrumented autonomous NAATs and other more advanced assays are to be successful for POC applications, then a non-instrumented controlled heating method could enable the above processes.

PATH and several others have previously demonstrated different methods for creating low-cost, non-instrumented, electricity-free heaters for isothermal amplification in traditional tube-in-solution and microfluidic applications.¹⁷⁻¹⁹ These heaters rely on exothermic chemical reactions, phase change materials (PCM), or a combination of both to provide the necessary heat and temperature control. These exothermic chemical methods hold advantages over other simple energy sources, like batteries (See Section 3.3).

Heat and temperature control for paper fluidics presents new challenges that current chemical heaters do not address. These challenges include interfacing the heater with a paper matrix, providing an even temperature distribution across a larger, flat interface, and controlling the timing of heat production to correlate with sample movement through the paper network. Herein, we demonstrate a heating method that combines an exothermic chemical heat source with PCM temperature control designed specifically for isothermal amplification in a paper device. We also show the simplicity, accuracy, low cost, and feasibility of a disposable, POC, chemically heated, NAAT device.

2. Background to Device Components

2.1 First chemical heaters for diagnostics

In 2010, PATH demonstrated a prototype device that successfully amplified malaria DNA with a loop-mediated isothermal amplification (LAMP) reaction in a micro PCR tube.¹⁹ The next prototype iteration was designed with a 10-ounce, double-wall, vacuum insulated housing (Foogo, Thermos®) and was transferred to several collaborators who have used it to successfully amplify DNA with their own isothermal amplification assays and targets.^{20,21}

Each of these devices was optimized with PCM which had a different melting temperature and amount of calcium oxide (CaO) to meet required product specifications.

Over the last two years, there has been continued growth and development in the field of chemical heaters for POC diagnostics. Hatano, et al. used locally sourced hand warmers in a commercially available Styrofoam™ box to successfully perform a LAMP reaction.¹⁷ More recently, Liu, et al. developed an exothermic heater that used a paper wick to control the heat output of the exothermic reaction and used a PCM to buffer against various ambient conditions.¹⁸ Liu's device used a magnesium-iron alloy chemical fuel with water and a paraffin-based PCM.

NINA refers to “non-instrumented nucleic acid amplification” and to a wide range of chemical heaters developed at PATH.²² There are several different embodiments of the NINA technology that warrant defining. The reusable-housing platform (RHP) has been designed to heat micro PCR tubes and takes advantage of a highly insulative and robust housing. We envision the RHP being marketed in a kit format with one housing and multiple disposable heater cartridges that can be simply dropped into the housing and activated.

The newest RHP NINA prototype was updated to work in a smaller 7-ounce vacuum-insulated housing (Foogo Thermos®: http://www.thermos.com/product_catalog.aspx?catCode=foog). These devices are designed to operate with a magnesium iron (MgFe) reaction rather than CaO (Section 2.2). They have also been updated with a new PCM to reduce the operating temperature for this particular isothermal amplification chemistry (44°C +/- 1°C for a duration of 30 minutes with a 15-minute warm-up time.) These RHP devices are designed to be robust and reusable through multiple cycles. The PCM is able to cycle through tens of thousands of thermal cycles (<http://www.puretemp.com/technology.html>) .

Another potential embodiment of the NINA technology is a single-use, disposable (SUD) device; this work focuses on a completely disposable device for isothermal amplification of nucleic acids under development in a project supported by the Defense Sciences Office (DSO) of the Defense Advanced Research Projects Agency (DARPA). The SUD format has been the main focus of recent design work (Section 3.2).

2.2 Exothermic reaction

There are currently five main exothermic chemical reactions being employed in existing commercial products (Table 1). These reactions have some basic characteristics in common that make them suitable for their usage; they are low cost, nontoxic, and easy to manufacture and package.

Over the last five years, PATH's engineering team has conducted in-depth empirical studies to investigate the potential use of sodium acetate, calcium oxide, and magnesium iron alloy in chemical heaters. Now, as a PATH/University of Washington (UW) collaboration, we have a thorough understanding of the attributes and best applications of each of these exothermic reactions. Sodium acetate, for example, produces heat through the crystallization of its super-saturated liquid form (heat of fusion), which is reversible by remelting the

crystals back into the liquid phase. This makes sodium acetate ideal for reusable products, but its relatively low heat output and limited peak temperature makes it a less-attractive option for assays requiring over 58°C or hold-over times longer than a few minutes (58°C is the melt temperature of sodium acetate trihydrate.) CaO is available in bulk at low prices and has a higher heat output per gram than sodium acetate, making it a good choice for single-use heaters. However, the heat output of bulk CaO varies significantly from batch to batch. This inconstancy is caused by natural variation in the limestone that CaO is typically derived from.²³ MgFe alloy, meanwhile, has approximately 12.6 times the heat output per mass of CaO and 55.8 times the output of sodium acetate (Table 1). Additionally, it is an engineered material with very consistent energy output (J/g) between batches that has been used for years as the primary heating source for the United States military's self-heating MREs (meals ready to eat).²⁴ It is, therefore, both inexpensive and in plentiful supply. For these reasons, all of our current work is focused on the use of MgFe alloy.

It is worth noting that although the iron/air reaction has a very high exothermic energy density (Table 1), it is unlikely to be the best choice of fuel for a NINA-type device due to difficulty in regulating the reaction.

The exothermic reaction of magnesium alone with H₂O is slower than practical for most commercial heating products or POC diagnostics; this is because of the formation of a semi-protective oxide layer that forms on the surface of the magnesium that inhibits it from continually reacting with the H₂O.²⁵ To overcome this limitation, a galvanic corrosion reaction is employed to speed up the reaction, increasing energy output.

The overall chemical formula for this oxidation reaction is as follows:

Galvanic corrosion is an electrochemical reaction that occurs between dissimilar metals under specific conditions. To achieve galvanic corrosion with the magnesium fuel, it is mechanically alloyed with iron.²⁴ This ensures that the metals are in direct electrical contact. When the powdered form of this alloy is submersed in an electrolyte solution, the metals form a galvanic couple with magnesium as the anode and iron as the cathode.

A simplified diagram (Figure 3) shows the general reaction pathways occurring during the galvanic corrosion of magnesium. Although the Mg (OH)₂ product normally forms the protective oxide film in this reaction, it breaks down in neutral to acidic environments and is vulnerable to attack by certain families of salts.²⁶ Using sodium chloride as an electrolyte, for example, breaks down the oxide film through a side reaction with the Mg (OH)₂, producing MgOHCl and allowing oxidation to continue unabated.

The oxidation of magnesium can be broken down into the following equations:

This gives an approximate theoretical heat of formation of $\text{Mg}(\text{OH})_2$ of 14.52 kJ per gram of Mg metal.

Any design using the MgFe reaction will need to account for the hydrogen gas that is released during the reaction (approximately 0.8 liters of hydrogen gas is released per gram of MgFe fully reacted.)

2.3 Development and availability of phase change material

As mentioned, both PATH's RHP heater and the heater demonstrated by Liu, et al. used a PCM as part of the system to control the maximum temperature achieved. PCMs are substances utilized for their latent heat at a specific melt temperature. Latent heat is the energy absorbed or released by the material as it undergoes a phase transition. While both phases are present (at the melting temperature of the PCM), assuming sufficiently high thermal conductivity, the system does not change temperature.

PCMs are currently used in many fields, including energy management for commercial and residential buildings, solar energy heat storage, and even some consumer products such as textiles.²⁸ To be most effective, a PCM should absorb a large amount of energy during phase change, change phase over a narrow temperature range, and be thermally conductive.^{29,30}

The growing industry for PCMs has created a need for innovative new products. A number of companies are currently addressing this market with a wide range of PCMs for many applications. Table 2 below shows some of the currently available materials that melt between 58°C and 68°C. This is a common temperature range specified for LAMP assays. These materials are nontoxic and biodegradable, which makes them ideal for use in a disposable device. Some companies are able to develop new PCMs to a particular specification for a fee.

A well-engineered and/or well-selected PCM allows a chemically heated device to consistently perform with unprecedented temperature control and tolerances. The melt temperature of a PCM can have a tighter tolerance, nearly infinite shelf life, and very little batch-to-batch variation (www.puretemp.com). The consistent melt temperature of the PCM and the way PCM is used in these prototypes helps mitigate, and even eliminate, any variation that would be caused by fluctuation in the exothermic reaction or inconsistencies that will inevitably arise in the manufacturing of devices.

3. Results to Date

The current PATH/UW team is engaged in a collaborative effort to develop an SUD NINA chemical heater for a two-dimensional paper network platform (MAD NAAT, for “multiplex autonomous diagnostic nucleic acid amplification test”).^{1,3,4} In this case, the amplification reaction occurs within a thin porous membrane, and the entire device must be a single-use disposable, so it is critical to minimize the size of the heater and its associated

insulation. We began the redesign process by creating some basic mathematical models and equations to understand the role each component plays in the overall system.

3.1 Mathematical modeling of fuel and phase change material requirements

Equations have been developed to begin describing the role of PCM in our device and how factors like acceptable ambient temperature range, device insulation, reaction temperature, and reaction power output profile affect the amount of PCM required to reliably hold the set temperature for the required period. The PCM in the device serves multiple roles, two of which are highlighted in Figure 4.

In the RHP platform, where the exothermic reaction produces heat at a much higher rate than the rate of system losses to ambient temperature, the temperature buffering role of the PCM is its primary function. The RHP is extremely well insulated, using an evacuated region that has been optimized to minimize radiant heat transfer as insulation for the majority of the heat loss area (everywhere besides the cap). Also, the RHP format currently adds all of the water to the exothermic material at once, creating a significant energy spike. Consequently, the volume of PCM that is needed to buffer the power output of the chemical reaction over time exceeds the volume that is required to allow operation under a range of ambient temperatures.

In a device designed to be fully disposable, designing the PCM to hold temperature is quite different. In order to keep costs down, the SUD device will likely have far less insulation than what is used with the RHP. In this case, the role of the PCM that accounts for ambient temperature range will have increased importance. Fortunately, the SUD device for heating a two-dimensional paper network (2DPN) format lends itself to exothermic reaction reagent control, which should require significantly less PCM than an RHP device for exothermic reaction dampening. Through the development of this disposable device, we have explored several promising methods for controlling the exothermic reaction. These methods can be used in conjunction with PCM heat storage to optimize the reaction.

Calculating the amount of phase change material needed for an arbitrary ambient temperature range—The minimum amount of fuel energy required is a function of test time, the difference between test temperature and lowest allowable ambient temperature, and the insulating properties of the device. Temporarily ignoring the transient period of bringing the device up to operating temperature (and that the amount of energy required depends on the ambient temperature), the device is simplified as a uniform-temperature heated zone surrounded by an insulating shell. Using these simplifications, the minimum amount of fuel energy required to account for heat loss from the device can be estimated as follows:

$$E_F = \left(\frac{T_T - T_L}{R_{sys}} \right) t \quad [1]$$

Where E_F = minimum fuel energy, T_T = assay target temperature, T_L = lower ambient limit, R_{sys} = device overall thermal resistance, and t = assay time. T_L is a device specification

corresponding to the lowest allowable ambient temperature for the device to function as designed. The high ambient limit is the highest allowable ambient temperature and is denoted by T_H .

At the high ambient temperature limit, the same amount of energy is produced by the fuel as at the low ambient temperature limit. Less energy is lost to the environment at high ambient temperatures than at low; the heat loss (P) can be described by:

$$P = \frac{\Delta T}{R_{sys}} \quad [2]$$

where ΔT is the difference between the assay temperature and ambient temperature. The total fuel energy produced at T_H is equal to the energy lost to the environment plus energy that needs to be stored by the PCM, E_{PCM} :

$$E_F = E_{heatloss} + E_{PCM} \quad [3]$$

The energy lost to the environment ($E_{heatloss}$) can be estimated at T_H by:

$$E_{heatloss} = \left(\frac{T_T - T_H}{R_{sys}} \right) t \quad [4]$$

Combining equations [1], [2], and [4], the energy that needs to be stored by the PCM for assays run in a range of ambient temperatures can be described by:

$$E_{PCM} = E_F - E_{heatloss} = \left(\frac{T_T - T_L}{R_{sys}} \right) t - \left(\frac{T_T - T_H}{R_{sys}} \right) t = \frac{t}{R_{sys}} [(T_T - T_L) - (T_T - T_H)] = \frac{t}{R_{sys}} (T_H - T_L) \quad [5]$$

Unsurprisingly, the amount of PCM required for the ambient range does not depend on test temperature but only on the allowable ambient temperature range, test time, and device insulation. The volume of PCM required for this purpose can be estimated by:

$$V_{PCM} = \frac{E_{PCM}}{L_{PCM} \rho_{PCM}} = \frac{(T_H - T_L)t}{R_{sys} L_{PCM} \rho_{PCM}} \quad [6]$$

where L_{PCM} = the latent heat of fusion of the PCM and ρ_{PCM} = the density of the PCM.

Additional PCM is required to account for uneven exothermic heat output over time and also to account for the transient period during which the device is heated up to the assay temperature from the ambient temperature. In order to arrive at this estimate, the simplifying assumption was made that the heater is of uniform temperature. Future heat transfer modeling will explore how differing geometry and thermal properties will affect PCM requirements. The operating environment was also simplified, considering an ambient temperature range and ignoring other factors (wind, etc.), which will also affect heat loss

3.2 Overview of variables for a heater design

In the following section, we describe some of the basic components and findings of the current SUD prototype designed for the MAD NAAT platform in order to demonstrate the feasibility of this technology. Detailed experimental results will be published elsewhere. The transition from a reusable heater for in-tube amplification (RHP) to an SUD for amplification in a two-dimensional paper substrate created some new design challenges including significantly reduced thermal insulation due to reduced cost and size requirements; limitations on heater and PCM orientation; chemical reaction activation; potential need for a delayed and controlled heat-up time; and finally the need for consistent, even heat over a larger area.^{1,3,4}

The current prototype (Figure 5) is integrated into a simple three-dimensional printed case (OBJET, Minneapolis, MN) that is capable of hinging open to allow loading of a sample paper membrane. The purpose of this prototype is to demonstrate the capability to heat a paper-based isothermal amplification reaction.

Some specifics to the current SUD prototype for the MAD NAAT platform are as follows.

The electrolyte in the exothermic reaction, sodium chloride (NaCl), can be either incorporated into the MgFe or it can be added to the water creating a saline solution. This latter approach is used in this prototype.

The PCM used is an eutectic mixture of myristic and stearic acids.³¹ Approximately 6 grams of PCM are used, providing about 1,080 joules of energy storage. The melt temperature of this mixture, measured by differential scanning calorimetry (DSC), is ~47.5°C (Figure 6). This melt temperature is very close to the ideal reaction temperature of our current isothermal assay and requires very little expense to make and use. Using compounds from Sigma Aldrich (95% purity) (www.SigmaAldrich.com) the per-test cost of this PCM is approximately \$0.12. Of course, this price could be reduced significantly if the compounds were purchased in a larger quantity.

Most of the available PCMs have low thermal conductivity compared to materials like water or aluminum. Several methods have been developed both internally and externally for improving the PCMs' ability to absorb and release heat. By increasing the thermal conductivity of the system, the temperature gradient in the volume of the PCM is minimized. Minimizing this gradient keeps the sample temperature close to the melt temperature, while the PCM furthest from the sample transitions from solid to liquid and back to a solid.²⁸⁻³⁰

The location of a heater for amplification in a 2DPN is limited. This configuration (Figure 5A) orients the heater above the paper network. This design holds several advantages over the alternative, with the heater below the network. It allows the entire length of the paper network to lie flat along the base of the device and maintains easy access to the exothermic reaction chamber for fluid delivery and activation. The exothermic fuel source is always in direct thermal contact with the PCM chamber, which increases thermal transfer efficiency. Similarly, the PCM is forced to maintain direct thermal contact with the target surface as it

melts. In the “top-heating” design, melted PCM flows downward toward the heated surface, preventing the formation of air pockets between the PCM and the target surface—a potential detriment to uniform heat transfer into the paper network.

Integrated into the housing of the SUD device is foam insulation. Current prototypes use a polyvinyl chloride (PVC) foam (www.mcmaster.com #9318K77) with thermal conductivity similar to foamed polystyrene. The PVC foam is easily machined and, therefore, more practical for in-house manufacture of prototypes. For this initial proof-of-concept SUD device, we specified a narrow ambient temperature range of 19°C to 24°C to replicate temperatures that are commonly found in environmentally controlled offices and laboratories.

In a practical device, consideration must be given to how the chemical reactants will be stored and how they will be brought together for the reaction to begin at the correct time. In the case of the MgFe reaction, two components must be kept separate in storage—the solid fuel and the liquid electrolyte solution.

The device shown in Figure 5 utilizes a thermoformed plastic blister sealed with a foil layer to store the electrolyte solution. These blister packs are easy to manufacture and are currently available for other microfluidic applications (microfluidic-chipshop.com). Using blister packs allows for very simple activation. By pressing the blister with appropriate force, the foil barrier fails and the electrolyte is delivered to the reaction chamber. Additionally, the user is not relied upon to measure the appropriate volume of electrolyte solution, reducing both user steps and the possibility of operator error.

Figure 6 shows the repeatability of the current prototype device as measured by a t-type thermocouple (OMEGA, 5SRTC-TT-T-40-36) located below the heater in the center of the sample area. The operational specifications for this device are 47.5°C $\pm$ 1.5°C with a 10-minute warm-up time.

3.3 Cost

The value of a chemical heater for a POC diagnostic device is at least in part dependent on keeping at a minimum the cost required to manufacture and integrate the heater into the device. The current design uses 0.25 g of MgFe alloy. At the current retail price of \$6.49 per pound of material, the cost of fuel is \$.0036 per test. The estimated retail cost of material for a single saline blister pack is ~\$.001, and the PCM costs ~\$.12 (Section 3.2).

For a basic cost analysis we compared the energy density of two types of standard alkaline batteries (AA and 9V) with the energy density of MgFe and CaO. The number of joules per gram per cubic centimeter and per \$.01 is taken into consideration (Figure 7).²

²The authors recognize that this is a simplistic model that looks purely at the characteristics of possible energy sources and does not account for the integration of these sources into an actual device. However, we feel these results make a strong initial case for the use of chemical heaters in single-use, disposable, diagnostic platforms.

4. Ongoing and Future Work

There are still significant design challenges that need to be addressed as the design moves towards a product that can be commercialized. The current SUD prototype is a reusable platform with a machined aluminum PCM housing and reaction chamber for the MgFe. Device components such as the PCM housing, saline blister pack, insulation, and exothermic fuel storage can be optimized for easier manufacturability and increased device performance.

We are also working to widen the ambient temperature range so the device can function beyond the controlled environment of the laboratory or an environmentally controlled office to the more demanding ambient conditions of many low-resource settings where a POC device can have real impact.

Finally, future prototypes will continue to be reduced in size where appropriate and in complexity where possible. These reductions will serve to widen the applicability of chemical heaters in the single-use, POC, diagnostic environment.

5. Conclusions

We have demonstrated the feasibility, simplicity, and low-cost chemical heating for paper fluidic devices. These heaters have also been demonstrated to produce consistent, tightly controlled temperatures in a range of conditions and specifications. Ongoing work focuses on optimizing heater design variables such as type, amount, and consistency of the exothermic material, type and amount of PCM, heat conductivity throughout the device, device configuration, insulation, and response to ambient temperature variability.

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References

1. Fridley GE, Le HQ, Fu E, Yager P. Controlled release of dry reagents in porous media for tunable temporal and spatial distribution upon rehydration. *Lab on a Chip*. 2012; 12(21):4321–4327. [PubMed: 22960691]
2. Lafleur L, Stevens D, McKenzie K, Ramachandran S, Spicar-Mihalic P, Singhal M, Arjyal A, Osborn J, Kauffman P, Yager P, Lutz B. Progress toward multiplexed sample-to-result detection in low resource settings using microfluidic immunoassay cards. *Lab on a Chip*. 2012; 12(6):1119–1127. [PubMed: 22311085]

3. Fu E, Ramsey SA, Kauffman P, Lutz B, Yager P. Transport in two-dimensional paper networks. *Microfluid Nanofluidics*. 2011; 10(1):29–35. [PubMed: 22140373]
4. Fu E, Liang T, Spicar-Mihalic P, Houghtaling J, Ramachandran S, Yager P. Two-dimensional paper network format that enables simple multistep assays for use in low-resource settings in the context of malaria antigen detection. *Anal Chem*. 2012; 84(10):4574–4579. [PubMed: 22537313]
5. Martinez A, Phillips S, Butte M, Whitesides G. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angewandte Chemie International Edition*. 2007; 46(8):1318–1320.
6. Carrilho E, Martinez AW, Whitesides GM. Understanding wax printing: a simple micropatterning process for paper-based microfluidics. *Anal Chem*. 2009; 81(16):7091–7095. [PubMed: 20337388]
7. Osborn JL, Lutz B, Fu E, Kauffman P, Stevens DY, Yager P. Microfluidics without pumps: reinventing the T-sensor and H-filter in paper networks. *Lab on a Chip*. 2010; 10(20):2659–2665. [PubMed: 20680208]
8. Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH. Microfluidic diagnostic technologies for global public health. *Nature*. 2006; 442(7101):412–418. [PubMed: 16871209]
9. Weigl B, Domingo G, LaBarre P, Gerlach J. Towards non- and minimally instrumented, microfluidics-based diagnostic devices. *Lab Chip*. 2008; 8(12):1999–2014. [PubMed: 19023463]
10. Lie P, Liu J, Fang Z, Dun B, Zeng L. A lateral flow biosensor for detection of nucleic acids with high sensitivity and selectivity. *Chem Commun*. 2012; 48(2):236–238.
11. Seal J, Braven H, Wallace P. Point-of-care nucleic acid lateral-flow tests. *IVD Technology Magazine*. Nov-Dec;2006 :41–51.
12. Cai, H. An inexpensive and simple nucleic acid dipstick for rapid pathogen detection. Conference Proceeding Washington, DC; Pathobiologics International Conference; August 22, 2006; Washington, D.C.. 2006.
13. Niemz A, Boyle DS. Nucleic acid testing for tuberculosis at the point-of-care in high-burden countries. *Expert Rev Mol Diagn*. 2012; 12(7):687–701. [PubMed: 23153237]
14. Tuberculosis diagnostic technology landscape: semi-annual update. UNITAID report. 2012
15. Aldous WK, Pounder JI, Cloud JL, Woods GL. Comparison of six methods of extracting mycobacterium tuberculosis DNA from processed sputum for testing by quantitative real-time PCR. *J Clin Micro*. 2005; 43(5):2471–2473.
16. Kulski JK, Pryce T. Preparation of mycobacterial DNA from blood culture fluids by simple alkali wash and heat lysis method for PCR detection. *J Clin Micro*. 1996; 34(8):1985–1991.
17. Hatano B, Maki T, Obara T, Fukumoto H, Hagsawa K, Matsushita Y, Okutani A, Bazartseren B, Inoue S, Sata T, Katano H. LAMP using a disposable pocket warmer for anthrax detection, a highly mobile and reliable method for anti-bioterrorism. *Jpn J Infect Dis*. 2010; 63(1):36–40. [PubMed: 20093760]
18. Liu C, Mauk MG, Hart R, Qiu X, Bau HH. A self-heating cartridge for molecular diagnostics. *Lab on a Chip*. 2011; 11:2686. [PubMed: 21734986]
19. LaBarre P, Gerlach J, Wilmouth J, Beddoe A, Singleton J, Weigl B. Non-instrumented nucleic acid amplification (NINA): instrument-free molecular malaria diagnostics for low-resource settings. *Conf Proc IEEE Eng Med Biol Soc*. 2010
20. Kubota R, LaBarre P, Singleton J, Beddoe A, Weigl BH, Alvarez AM, Jenkins DM. Non-instrumented nucleic acid amplification (NINA) for rapid detection of *Ralstonia solanacearum* race 3 biovar 2. *Biol Eng Trans*. 2011; 4(2):69–80.
21. Curtis KA, Rudolph DL, Nejad I, Singleton J, Beddoe A, Weigl B, LaBarre P, Owen SM. Isothermal amplification using a chemical heating device for point-of-care detection of HIV-1. *PLoS One*. 2012; 7(2):e31432. [PubMed: 22384022]
22. LaBarre P, Hawkins K, Gerlach J, Wilmouth J, Beddoe A, Singleton J, Boyle D, Weigl B. A simple, inexpensive device for nucleic acid amplification without electricity—toward instrument-free molecular diagnostics in low-resource settings. *PLoS One*. 2011; 6(5):e19738. [PubMed: 21573065]
23. [Accessed January 15, 2013] Merck Index of Chemicals and Drugs. Available at: <http://library.dialog.com/bluesheets/html/bl0304.html>
24. Kuhn, WE.; Hu, KH.; Black, A. Flexible Electrochemical Heater. 4,522,190. Jun 11. 1985

25. Bell, WL.; Copeland, RJ.; Schultz, AL. Application of New Chemical Heat Sources, Phase I. US Army and Biological Chemical Command, Publishers, TR-01/004; 2001.
26. Shaw, BA. Corrosion resistance of magnesium alloys. In: Hertfordshire, U Hitchin, editor. ASM Handbook, Volume 13A: Corrosion, Fundamentals and Protection. ASM International; 2003. p. 692-696.
27. Chase MW. J Phys Chem Ref Data, Monograph 9. 1998:1–1951. NIST-JANAF Thermochemical Tables, Fourth Edition.
28. Farid MM, Khudhair AM, Razack SA, Al-Hallaj S. A review on phase change energy storage: materials and applications. *Ener Conv and Mgmt.* 2004; 45(9):1597–1615.
29. Ettouney H, Alatiqi I, Al-Sahali M, Al-Ali S. Heat transfer enhancement by metal screens and metal spheres in phase change energy storage systems. *Renewable Energy.* 2004; 29:841–860.
30. Yavari F, Fard HR, Pashayi K, Rafiee MA, Zamiri A, Yu ZZ, Ozisik R, Borca-Tasciuc T, Koratkar N. Enhanced thermal conductivity in a nanostructured phase change composite due to low concentration graphene additives. *J Phys Chem.* 2011; 115(17):8753–8758.
31. Costa MC, Sardo M, Rolemberg MP, Ribeiro-Claro P, Meirelles AJA, Coutinho JAP, Krahenbuhl MA. The solid-liquid phase diagrams of binary mixtures of consecutive, even saturated fatty acids: differing by four carbon atoms. *Chem and Phys Lipids.* 2009; 157(1):40–50. [PubMed: 18996101]

Figure 1. Cross section of the non-instrumented nucleic acid amplification reusable-housing platform

Visible are two of the three sample wells. Surrounding these wells is the PCM chamber; the exothermic reaction takes place below the PCM inside the bottom of the stainless-steel, vacuum-insulated housing.

Figure 2. Ambient temperature testing of the reusable-housing platform device

The prototype functioned within specification for a tested ambient temperature range of 22°C to 38°C, N=18. For this experiment, three devices were first conditioned in an environmental control chamber for sufficient time to allow the device to stabilize at the test ambient temperature. (NOTE: The start temperature at 0 minutes shows the temperature of the conditioned device and the environmental chamber.) This graph demonstrates how precisely and repeatably a chemically heated device can operate.

Figure 3. Reaction pathways occurring during the galvanic corrosion of magnesium

This simplified diagram shows the reaction pathways occurring during the galvanic corrosion of the MgFe alloy. The Mg is oxidized and water is reduced, facilitating the reaction between Mg^{2+} and $2(OH)^-$ to form $Mg(OH)_2$ and produce heat. The magnesium metal is oxidized to Mg^{2+} and water at the cathode is reduced to form hydroxyl ions (OH^-) and hydrogen gas (H_2).

Figure 4. Two roles of phase change material in an isothermal heating application

A: When the device is operated at the upper ambient temperature limit, less heat is lost to the environment as described by $P = \text{heat loss} = T/R_{\text{sys}}$, resulting in “extra” fuel in the device. The PCM needs to store the energy created by this “extra” fuel in order to keep the test area from exceeding the temperature set point when the device is operated at a higher temperature than the lower ambient limit. Additionally, more energy is required to bring the system up to the test temperature at low ambient temperatures than at high ones. **B:** PCM serves to buffer uneven energy production over time. For example, the previous RHP device delivers all of the water needed for the chemical reaction at once, creating a significant spike in energy during the early part of the assay. The energy from this spike is stored in the PCM to be recovered after all of the exothermic material has reacted. The magnitude of this spike can be reduced by regulating the exothermic reaction by controlling the water supply or a similar method.

Figure 5.

A: An isometric rendering of the current single-use, disposable prototype design for running an isothermal amplification assay in paper.

B: Cross sectional view of the current single-use, disposable prototype.

Figure 6. Heater performance

The solid line shows the average temperature value of 12 runs at the sample interface for each time point. The acquisition rate was one data point per second starting from reaction activation. The dotted lines represent the average value plus and minus one standard deviation.

Figure 7. Energy analysis

This chart demonstrates the differences between the joules stored in batteries as compared to exothermic reaction materials.

“Per cost (\$0.01)” = kilojoules available in \$0.01 worth of material or kilojoules/battery cost in cents. “Per Volume (cc)” = kilojoules available in a cubic cm of material or battery volume (cc)/kilojoules. “Per Mass (g)” = kilojoules available in a gram of material or battery mass (g)/kJ.

The MgFe not only outperforms standard batteries in cost; it also has significantly higher energy density, potentially reducing device size.

Table 1
Existing commercial products using exothermic heat reactions

Reactants	Reaction Inputs Equation	Exothermic Heat Output	Sample Consumer Product*
Calcium Oxide + H ₂ O	CaO + H ₂ O	1.15 kJ/g	Hot Can (www.hot-can.com)
Calcium Chloride + H ₂ O	CaCl ₂ + H ₂ O	0.73 kJ/g	Rocket Fuel Coffee (http://rocketfuel.uk.com)
Iron + Air (O ₂)	Fe + (3) O ₂	29.52 kJ/g	HotHands hand warmers (www.hothandsdirect.com)
Sodium Acetate	Na ⁺ C ₂ H ₃ O ₂	0.26 kJ/g	Heat Wave reusable hand warmers (www.bentgrassconcepts.com)
Magnesium Iron Alloy + H ₂ O	MgFe + H ₂ O	14.52 kJ/g	HeaterMeals (www.heatermeals.com) Field ration heater for meals ready to eat (MRE) (www.innotechproductsltd.com)

* Not an exhaustive list of manufacturers or products.

Table 2
Commercially available phase change materials

Melt Temperature (°C)	Name	Manufacturer	Latent Heat of Melting (J/g)
68	PureTemp 68	Entropy Solutions	198
65	RT 65	Rubitherm	152
63	PureTemp 63	Entropy Solutions	199
62	PlusICE® A62	PCM Products, Ltd.	145
61	PureTemp 60	Entropy Solutions	230
61	RT 62	Rubitherm	146
60	PlusICE® A60	PCM Products, Ltd.	145
60	RT 60	Rubitherm	144
58	PCM-HS58P	RGEES, LLC	250
58	PureTemp 58	Entropy Solutions	237
58	RT 58	Rubitherm	178
58	PlusICE® A58	PCM Products, Ltd.	132