HOT Diagnostic Technologies

Low-cost, point-of-care nucleic acid amplification using chemical heat to replace traditional heat sources

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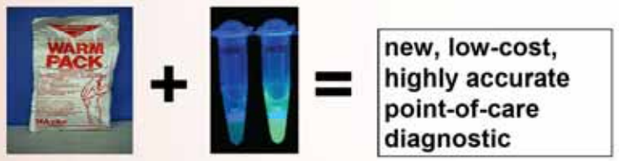
Introduction

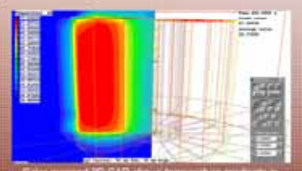
Many infectious diseases that impact global health are most accurately diagnosed through nucleic acid (NA) amplification and detection. However, low-cost, highly accurate, disposable nucleic acid amplification tests (NAATs) are not accessible to underserved populations in low-resource settings.

We have successfully demonstrated the technical feasibility of several components of a new, low cost, disposable diagnostic platform that can be used for detection of a wide variety of infectious diseases. We have combined two unrelated technologies. The first technology is exothermic chemical heating, a mature, commercially available technology found in "ready-to-eat" meals and skier's hand warmers. We have thermally coupled exothermic reactions to phase-change materials (PCMs) to absorb latent heat at constant temperature. The resultant heating unit can produce consistent, constant-temperature heat largely independent of external conditions. The second technology is loop-mediated isothermal amplification LAMP, a novel, highly accurate, DNA amplification technology. The proposed platform will function as a self-contained, point-of-care device suitable for low-resource settings, refugee camps, home use, as well as disaster response.



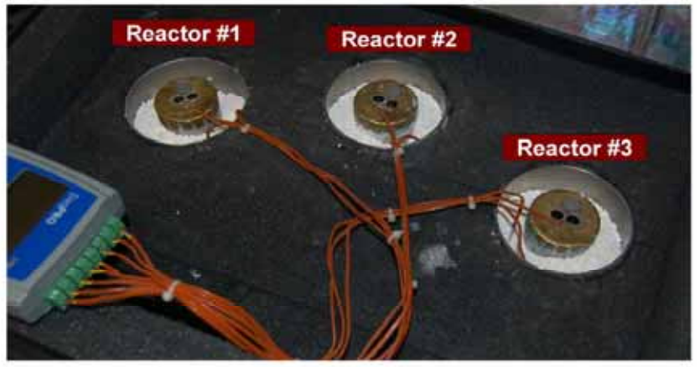
CFD Methods

PATH conducted a feasibility study of an exothermically heated, PCM-moderated, diagnostic platform using computational fluid dynamics (CFD). The work included a characterization of candidate exothermic reactions and PCMs. 3D computer aided design (CAD) geometries of the platform were created and numerically analyzed for six unique configurations of exothermic reaction and PCM using a commercially available CFD software package, PHOENICS by CHAM Ltd. Of the configurations assessed, the best performing configuration used 20 g calcium oxide (CaO) combined with 100 g water occupying a tubular space with an outer diameter (OD) of 22 mm, an inner diameter (ID) of 17 mm and a height (h) of 67 mm. Heat output of this CaO mixture was assumed to be 650,000 Watts/m^3 based upon empirical data. The exothermic reaction surrounds a paraffin-based PCM with melt temperature 62°C which occupies a cylindrical space with OD = 16 mm and h = 67 mm. The simulated LAMP reaction volume was 50 uL in a standard 5 mm diameter 200 uL cuvette located at the center of the PCM. The entire configuration was simulated enclosed within a high-density open cell urethane foam.


Color-mapped 3D CAD shows temperature gradients in the CFD-simulated diagnostic reaction chamber

Prototype Construction

PATH created several identical insulated prototype reaction chambers using the leading CFD geometry as a guide. The reaction chamber is constructed of stainless steel tubes welded to stainless steel plate with formed brass sheet lids. Each reactor is designed to hold 4 standard 200uL cuvettes. For insulation, the reactors are encased in foam and housed in a hard plastic case. While the geometry and materials are non-optimized with respect to thermal mass, these devices allowed empirical assessment of multiple CaO / PCM configurations. Through these experiments we determined that 77 g CaO combined with 17 g water and buffer provides sufficient heat energy to drive the LAMP isothermal NA amplification reaction. In order to reach and maintain 62°-65°C for the required 45 minutes, the exothermic reactants are thermally coupled to a proprietary PCM (Entropy Solutions, Inc.) designed to melt at 65°C. The PCM absorbs and releases relatively large amounts of latent heat at relatively constant temperatures. These PCMs are made from a green technology in that underutilized bio-based oils are converted into PCMs.



Laboratory Methods

Using the prototype reactors described above, PATH conducted a feasibility study using clinically relevant dilution panels of malaria DNA. For demonstration purposes, the LAMP reaction protocol described by Poon et al, 2006 was used to amplify genomic *Plasmodium falciparum* malaria DNA. Duplicate samples of DNA and primers (Eiken LoopAmp DNA amplification Kit PN LMP206) were simultaneously run in the three reaction chambers while another set was mirrored in a PE Geneamp Thermocycler 9600 as a control. A multichannel digital thermometer (DaqPRO 5300 Data Recoder) was used to record the thermal profile of each reaction chamber after the exothermic reaction was initiated. Cuvettes containing the LAMP mastermix were introduced when the thermocouples temperatures read 62°C. Separate tests were run for turbid visual detection and fluorescent detection (fluorescent detection reagent added to mix). Results from the dilution panel (approximate number of parasite genomes/mL: 5000, 500, 50, 5, and 1) from both the experimental reactor set and control set were compared to the no template negative control as well as a water only control.

Results

Dilution	gDNA (ng/uL)	Picograms/ uL	Approx number parasite genomes/uL	NINA MT3000 @ 63°C ± 1°C	PE Geneamp Thermo Cycler 9600 @ 63°C ± 0.75°C
A	0.1	100	5000		
B	0.01	10	500		
C	0.001	1	50		
D	0.0001	0.1	5		
E	2.00E-05	0.02	1		
-	NTC	0	0		

Dilution	gDNA (ng/uL)	Picograms/ uL	Approx number parasite genomes/uL*	NINA MT3000 @ 63°C ± 1°C	PE Geneamp Thermo Cycler 9600 @ 63°C ± 0.75°C
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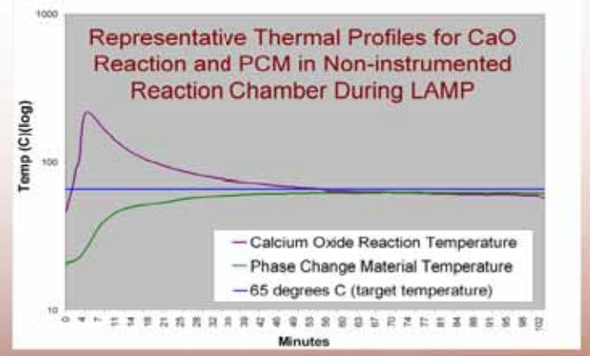

Support for this project was provided through funding from private foundations and individual donors to the Health Innovations Portfolio at PATH, as well as the United States Agency for International Development through the HealthTech program. The views expressed by the authors do not necessarily reflect the views of the funding agency.

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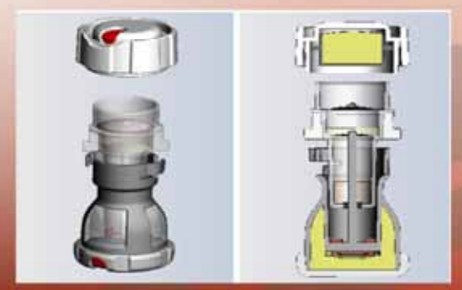
Conclusion

We have achieved the first complete, non-instrumented NAAT using a CaO heat source thermally linked to a proprietary phase-change material. These two components alone maintain a thermal profile suitable for a qualitative LAMP diagnostic assay. Starting with CFD analysis, we identified nominal geometry for the exothermic reaction chamber, PCM chamber, and thermal insulation. Using this model, we designed and fabricated an alpha prototype assay platform. We have verified the function of this multiple-pathogen-capable platform with both fluorescent and visual turbidity indications using samples spiked with clinically relevant dilutions of malaria DNA. Next steps will focus on the development of a smaller, optimized device and validation using clinical samples. We will also advance technologies necessary to enable a disease-specific configuration of this technology with LAMP kit on board in a stable, dry format.


Disease specific configuration of the exotherm / PCM technology with LAMP kit on-board in stable, dry format.