SUPPLEMENTARY INFORMATION

Smartphone-based Mobile Detection Platform for Rapid Molecular Diagnostics and Spatiotemporal Disease Mapping

Jinzhao Song[a], Vikram Pandian[a], Michael G. Mauk[a], Haim H. Bau[a], Sara Cherry [b], Laurence C. Tisi[c] and Changchun Liu[a,*]

[a] Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

[b] Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

[c] ERBA Molecular, Ely, Cambridgeshire CB7 4EA, United Kingdom

[*] **Corresponding author**

Dr. Changchun Liu

Department of Mechanical Engineering and Applied Mechanics

University of Pennsylvania

210 Towne Building

220 South 33rd St.

Philadelphia, Pennsylvania 19104-6315, USA

Phone: (215)746-2848

E-mail: lchangc@seas.upenn.edu

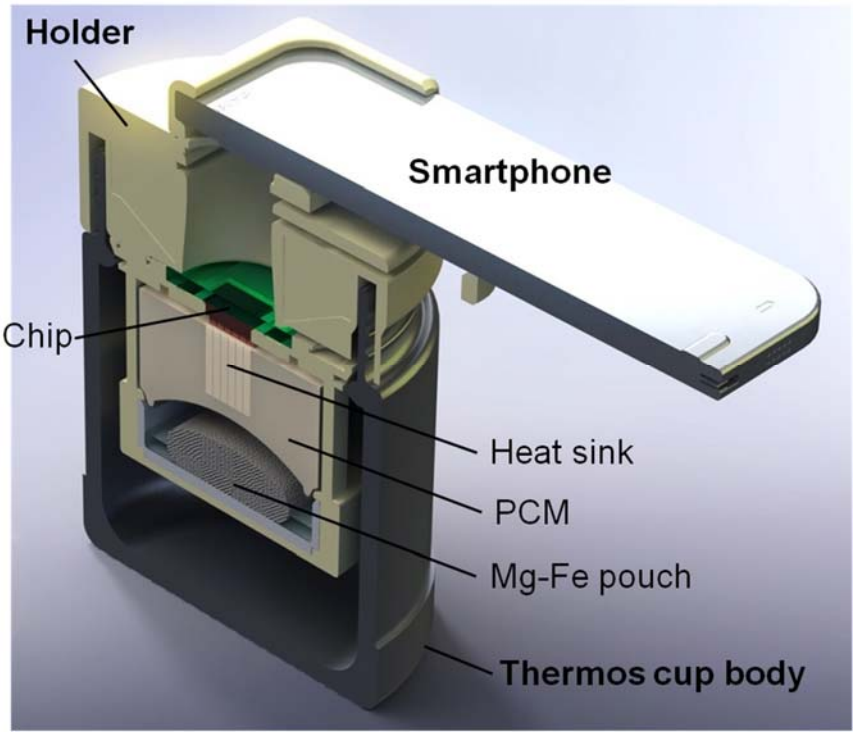
Supplementary Information

Supplementary File	Title
Table S1	LAMP primers' sequences for ZIKV and HIV detection
Figure S1	A cross-sectional view of the smart-connected cup
Figure S2	Detailed screenshots of our smartphone interface
Figure S3	Working principle of BART-LAMP assay
Figure S4	Real-time monitoring of RT-LAMP amplification on a benchtop thermal cycler operating at a fixed temperature
Figure S5	The effect of pyrophosphatase on BART-LAMP emission intensity
Video S1	An animation illustrating the various components of the smart cup
Video S2	Real time smartphone – based detection of BART-LAMP emission from a multifunctional amplification chip. The various reaction chambers, from top to bottom, contain 500, 50, 5, and 0 (no target control) PFU of ZIKV in a urine sample.
Note S1	Working principle of BART-LAMP assay
Note S2	Benchtop RT-LAMP ZIKV detection

Table S1: The sequences of LAMP primers for ZIKV and HIV and their concentrations in the BART-LAMP assay.

	Primer name	**Sequence (5' to 3')**	**Concentration (μM)**
LAMP primer set specific for ZIKV detection	F3	AGTTCAAGGACGCACATGC	0.2
	B3	AACGCTGCGR*TACACAAG	0.2
	FIP	GCCTCY*AGAGCTCCAGCAAG-AGGCAAACTGTCGTGGTTC	1.6
	BIP	GTGCAAAGGGAAGGCTGTCCTC-GAGTATGACACGCCCTTCAA	1.6
	Loop F	CTGCTCCTTCTTGACTCCCTA	0.8
	Loop B	TGGCCACTTGAAATGTCGC	0.8
LAMP primer set specific for HIV detection[1]	F3	ATTATCAGAAGGAGCCACC	0.2
	B3	CATCCTATTTGTTCCTGAAGG	0.2
	FIP	CAGCTTCCTCATTGATGGTTTCTTTTTAACAC CATGCTAAACACAGT	1.6
	BIP	TGTTGCACCAGGCCAGATAATTTTGTACTGGT AGTTCCTGCTATG	1.6
	Loop F	TTTAACATTTGCATGGCTGCTTGAT	0.8
	Loop B	GAGATCCAAGGGGAAGTGA	0.8
*R and Y indicate mixtures of primers in equal proportions, where R=A, G and Y=C, T			

(a)



(b)

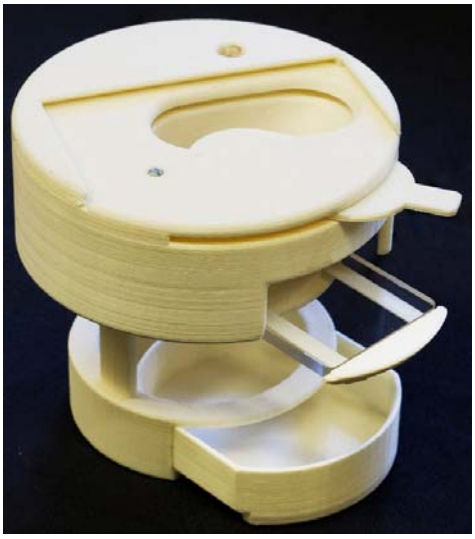
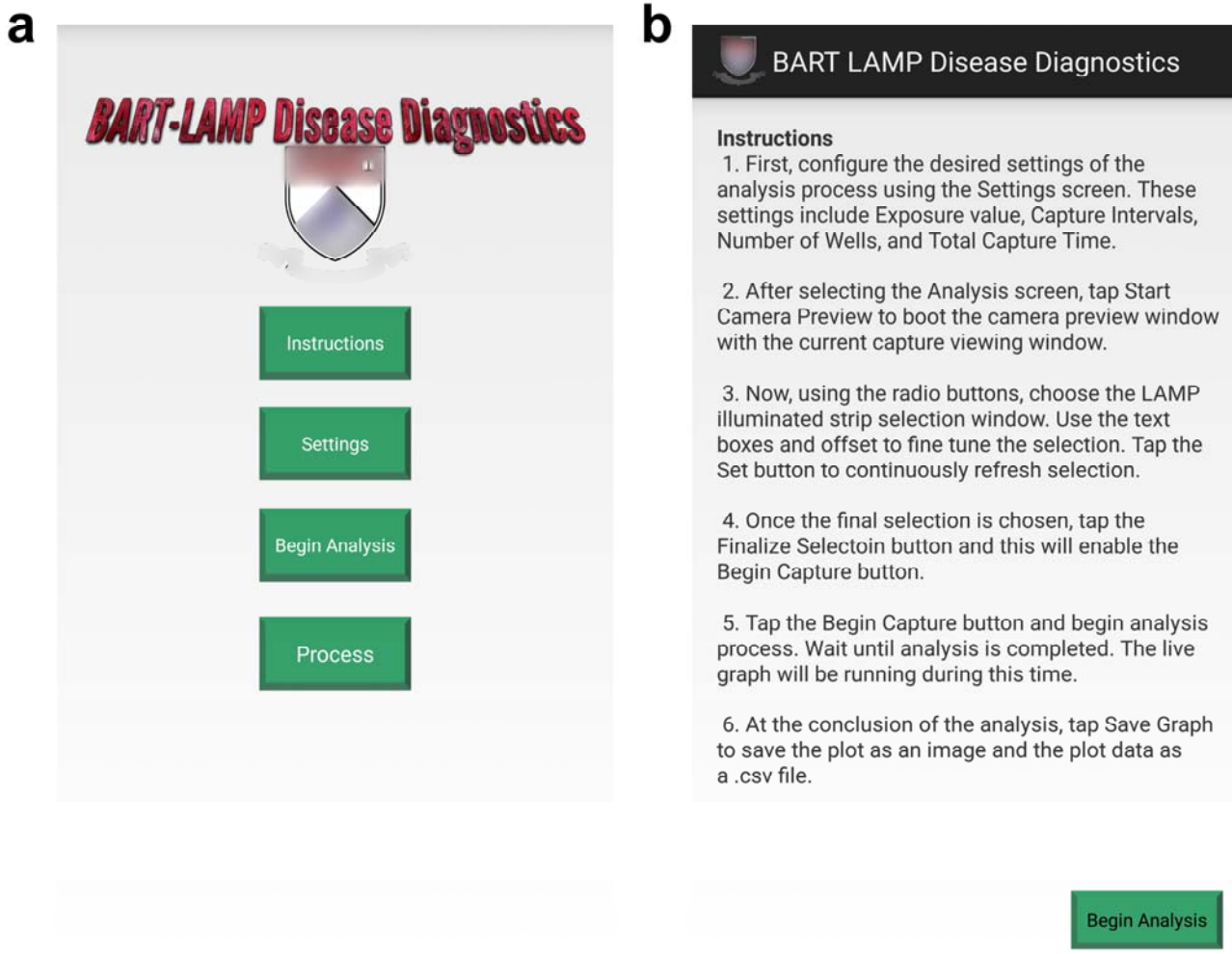
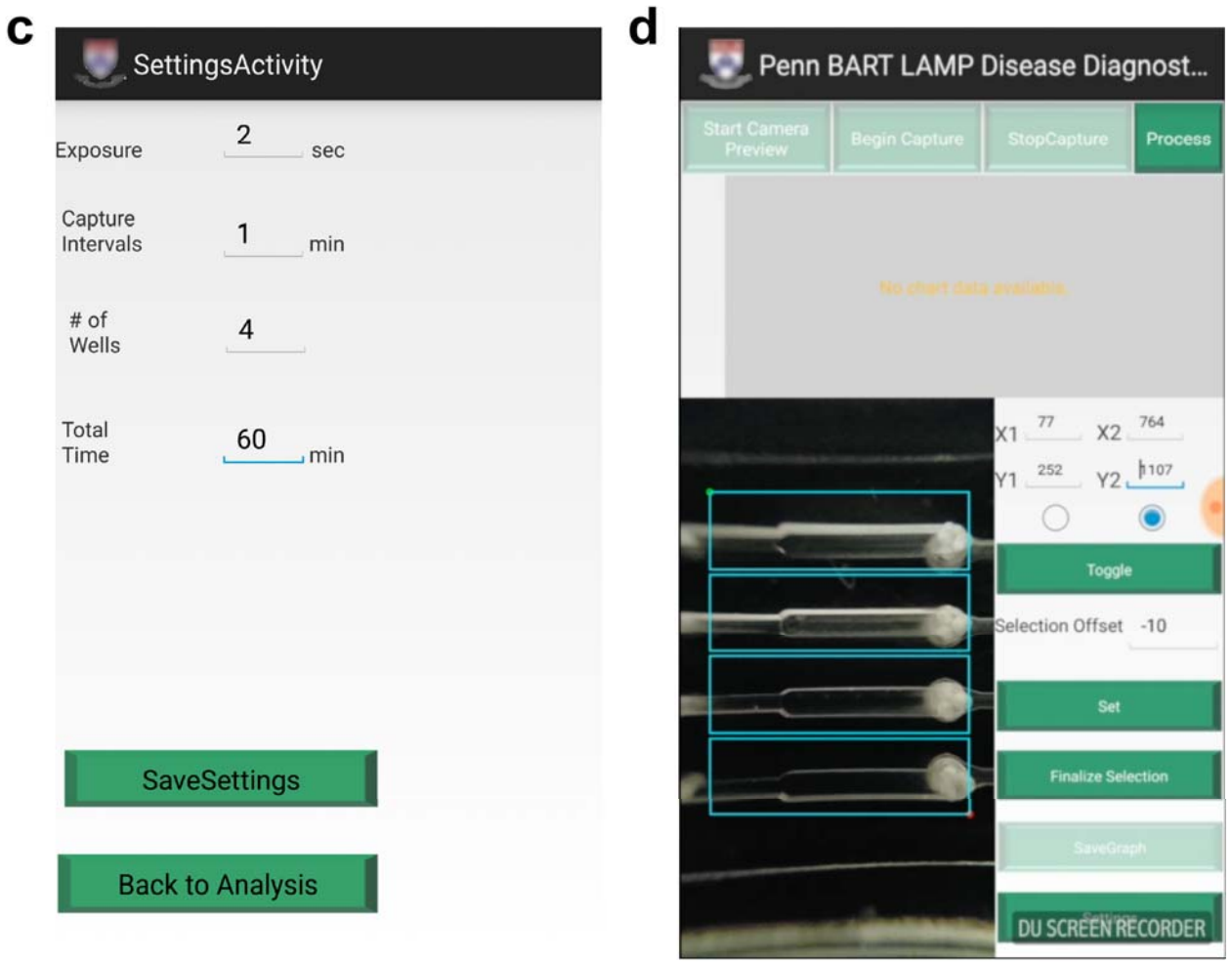


Figure S1: Smart-connected cup. (a) A cross-section of our smart-connected cup. Our smart-connected cup consists of a thermos cup body, a 3D-printed adaptor, and a smartphone. (b) A photo of 3D printed holder.


a
BART-LAMP Disease Diagnostics
Instructions
Settings
Begin Analysis
Process
b
BART LAMP Disease Diagnostics
Instructions
1. First, configure the desired settings of the analysis process using the Settings screen. These settings include Exposure value, Capture Intervals, Number of Wells, and Total Capture Time.
2. After selecting the Analysis screen, tap Start Camera Preview to boot the camera preview window with the current capture viewing window.
3. Now, using the radio buttons, choose the LAMP illuminated strip selection window. Use the text boxes and offset to fine tune the selection. Tap the Set button to continuously refresh selection.
4. Once the final selection is chosen, tap the Finalize Selectoin button and this will enable the Begin Capture button.
5. Tap the Begin Capture button and begin analysis process. Wait until analysis is completed. The live graph will be running during this time.
6. At the conclusion of the analysis, tap Save Graph to save the plot as an image and the plot data as a .csv file.
Begin Analysis


c
SettingsActivity
Exposure
2
sec
Capture Intervals
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min
# of Wells
4
Total Time
60
min
SaveSettings
Back to Analysis
d
Penn BART LAMP Disease Diagnost...
Start Camera Preview
Begin Capture
StopCapture
Process
No chart data available.
X1
77
X2
764
Y1
252
Y2
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Toggle
Selection Offset
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Set
Finalize Selection
SaveGraph
DU SCREEN RECORDER

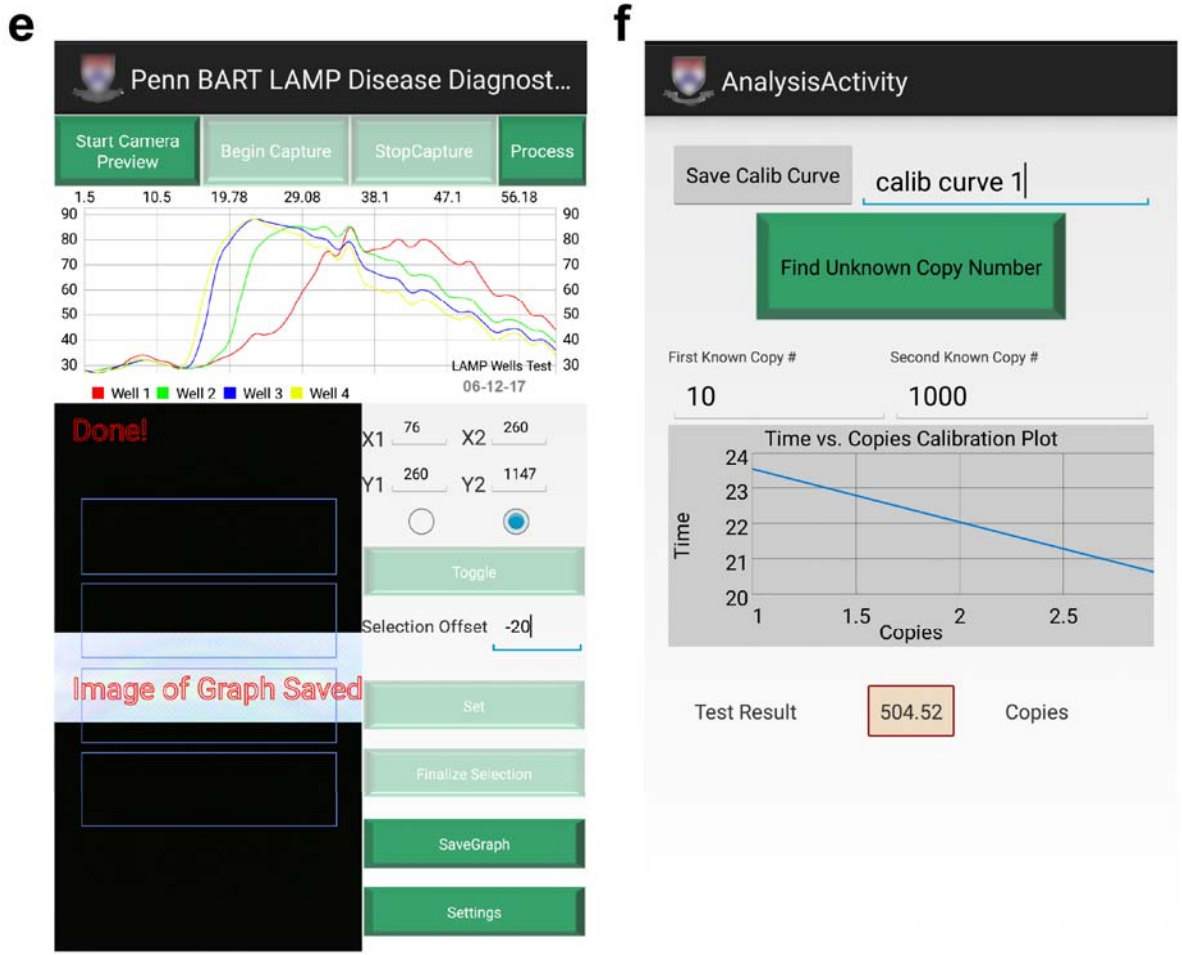

Figure S2: Screenshots of our smartphone user interface. (a) Main menu, (b) Instructions, (c) Parameter settings, (d) Alignment of detection regions of chip, (e) Signal read-out, dataacquisition and logging , and uploading data and GPS coordinates, and (f) Target quantification, and calibration curve archiving.

S1: The working principle of the BART-LAMP assay

Gandelman *et al*.[2] combined continuous enzymatic luminometric detection of inorganic pyrophosphate assay (ELIDA)[3] with nucleic acid amplification assay to form the Bioluminescent Assay for Real-Time--Loop Mediated Isothermal Amplification (BART-LAMP). The BART-LAMP assay reports continuously, through bioluminescent output, on the concentration of inorganic pyrophosphate (PPi) produced during isothermal amplification (Equation 1, **Figure S3**). The enzyme ATP sulfurylase (Equation 2, **Figure S3**) converts the inorganic pyrophosphate (PPi), generated during isothermal amplification, into ATP, which in turn fuels luciferin to produce light (Equation 3, **Figure S3**), enabling one to monitor the reaction rate in real time.

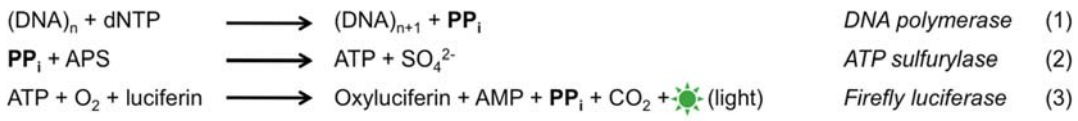

Figure S3: Biochemical reactions describing LAMP amplification (1), PPi conversion into ATP (2), and bioluminescent emission production (3).

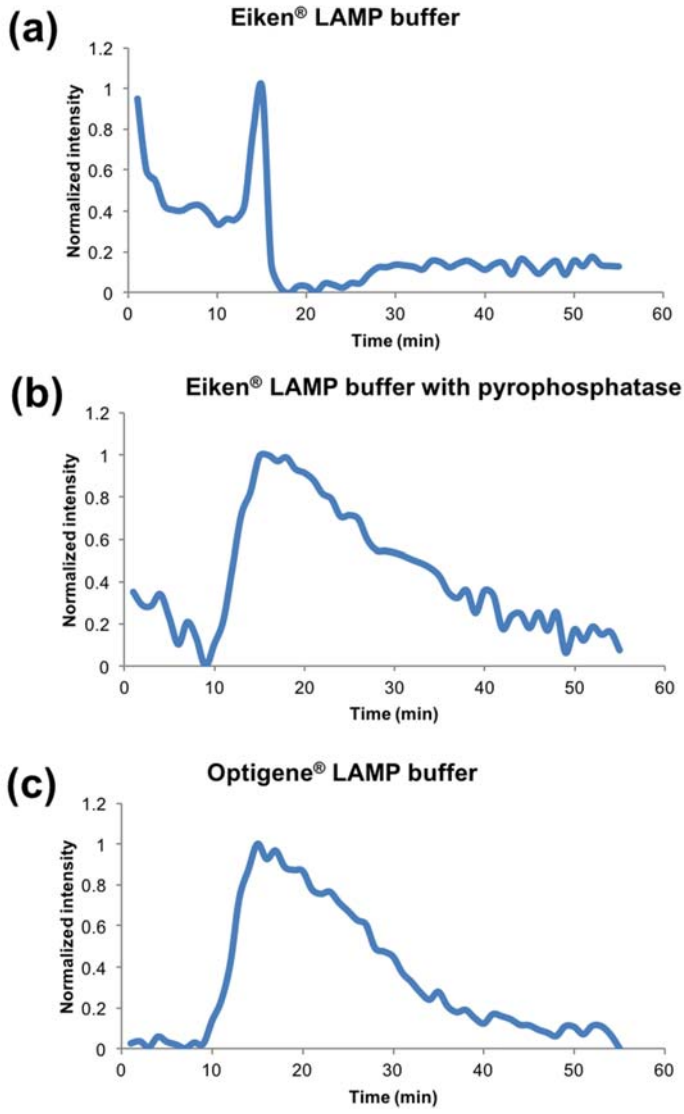

Figure S4: The effect of Pyrophosphatase on the BART-LAMP assay emission. (a) Real time BART-LAMP emission with Eiken® LAMP reaction buffer that does not contain pyrophosphatase. (b) Real time BART-LAMP emission with Eiken® LAMP reaction buffer with 1 U of pyrophosphatase. (c) Real time BART-LAMP emission with Optigene® LAMP reaction buffer that contains pyrophosphatase. The BART-LAMP curves are obtained in the presence of 500 PFU of ZIKV in each reaction volume. The data was collected by smartphone but without subtracting initial background.

S2: Benchtop RT-LAMP Amplification.

Zika viral RNA was extracted with Qiagen Viral RNA mini kit (QIAGEN, Valencia, CA), following manufacturer's recommendations[4]. In addition to the ZIKV primers (**Table S1**) and template, the RT-LAMP reaction mixture (15 µL) included: 9 µL of OptiGene Isothermal Master Mix ISO-100 (OptiGene, U.K.), 2 U of AMV reverse transcriptase (Invitrogen, Carlsbad, CA), and 0.5 µL of EvaGreen fluorescent dye (Biotium, Hayward, CA). Amplification was carried out and monitored with Peltier Thermal Cycler PTC-200 (Bio-Rad DNA Engine, Hercules, CA) at 63 °C. Fluorescence emission intensity data were collected once every minute for 60 min.

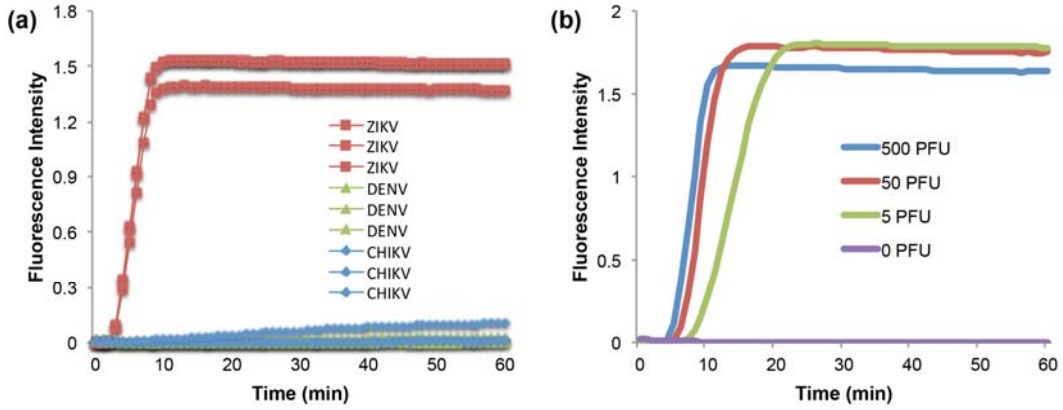

Figure S5: Real-time monitoring of LAMP amplification on a benchtop thermal cycler operating at a fixed temperature (63 °C). (**a**) Real-time monitoring of ZIKV LAMP with samples containing ZIKV, DENV and CHIV to evaluate ZIKV LAMP primers' specificity. Each reaction volume contains 50,000 PFU of ZIKV, DENV, and CHIKV. (**b**) Real-time monitoring of RT-LAMP assay of 500 PFU, 50 PFU, 5 PFU, and 0 PFU (negative control) of ZIKV per reaction volume.

References

(1) Curtis, K. A.; Rudolph, D. L.; Owen, S. M. *J. Virol. Methods* **2008**, *151*, 264-270.

(2) Gandelman, O. A.; Church, V. L.; Moore, C. A.; Kiddle, G.; Carne, C. A.; Parmar, S.; Jalal, H.; Tisi, L. C.; Murray, J. A. H. *PLoS One* **2010**, *5*.

(3) Nyren, P.; Lundin, A. *Anal. Biochem.* **1985**, *151*, 504-509.

(4) https://www.qiagen.com/us/shop/sample-technologies/rna/viral-rna/qiaamp-viral-rna-mini-kit/ - orderinginformation (accessed March 2, 2018).