# RAPID MULTIPLEX DETECTION OF VIRAL PATHOGENS IN WHOLE BLOOD USING MICROFLUIDIC SAMPLE PROCESSOR AND SMARTPHONE-LINKED HANDHELD INSTRUMENT

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## ABSTRACT

An integrated system for rapid multiplex of whole blood samples for simultaneous detection of Zika, Dengue (serotypes 1 and 3), and Chikungunya is demonstrated using a smartphone assisted detection device and microfluidic sample processor. The microfluidic processor stores all required reagents for sample lysis as well as LAMP master mix and partitions the final solution into 6 compartments for detection of 4 virus assays plus two experimental controls. With a handheld detection instrument that provides uniform 65°C heating, LED illumination, and a smartphone camera for image gathering, the multiplex assay is detected through LAMP-generated fluorescence within the distinct compartments. This system achieves detection limits of live virus spiked into whole blood through the ranges of $2x10^5$ – $6x10^6$ PFU/ml and lacks cross reactivity between assays while providing a pipette free operation with no additional equipment.

## KEYWORDS

Biosensor, point-of-care test, virus detection, LAMP, smartphone integration, lab on a chip, arbovirus

## INTRODUCTION

The emergence and resurgence of mosquito-borne viruses such as Zika, Chikungunya, and Dengue have heightened global health concerns. These arboviruses, spread through mosquitos, often co-circulate, causing overlapping symptoms that complicate clinical diagnosis[1]. Despite one being the most prevalent arboviruses each year, Zika does not have any available vaccine[2]. Chikungunya's first vaccine, a live attenuated virus vaccine, was only recently approved in 2023[3]. While these diseases often resolve with supportive treatment, early detection is important for prevention of severe manifestations which can become deadly, cause long term pain, or cause risk for fetal health due to Dengue, Chikungunya, and Zika respectively[4].

As with most infectious diseases, rapid and accurate point-of-care (POC) diagnostic methods are paramount for controlling outbreaks, enabling timely treatment, and improving patient outcomes. The World Health Organization has set a list of criteria for an ideal POC diagnostic with the acronym ASSURED emphasizing accuracy, accessibility, and affordability[5]. Currently, polymerase chain reaction (PCR) is the gold standard for viral detection due to its high sensitivity and specificity. However, it requires advanced laboratory infrastructure, trained personnel, and precise temperature cycling requiring bulky and expensive machinery, limiting its feasibility in resource-constrained settings.

Isothermal nucleic acid amplification methods are a promising alternative technique for POC diagnostics. Unlike PCR, loop-mediated isothermal amplification (LAMP) operates at a constant temperature (65°C), allowing for use of small and inexpensive heaters for portable use. Additionally, its use of the Bst polymerase, which is effective against tissue enhances robustness against interference, reducing false negatives. These features make LAMP particularly suitable for whole-blood testing without extensive preprocessing. Previously, we demonstrated Zika virus detection using a smartphone-based system with separate modules for sample preparation and readout, showcasing the potential of LAMP-based diagnostics for accessible and reliable POC testing [1].

## SYSTEM DESIGN

### Injection Molded Microfluidic Cartridge

Building upon prior work, we developed a field ready, smartphone-assisted detection system featuring a low-cost injection molded cartridge seen in **Figure 1**. The cartridge consolidates previously separate functions into a single unit: enabling sample lysis, reagent mixing, and distribution of the mixture into six reaction compartments using a hand operated threaded syringe, thus eliminating the use of laboratory equipment including pipettes or motorized syringes. The cartridge integrates a simple finger-prick blood collection mechanism, making it ideal for on-site testing in resource-limited environments.

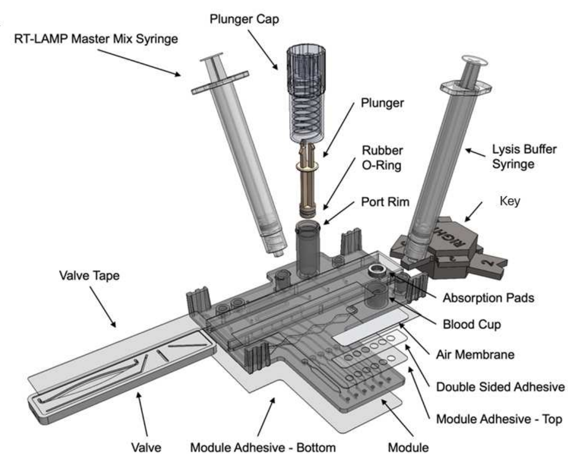


***Figure 1**: Labelled schematic of microfluidic cartridge. The valve is moved through the central channel to connect different microfluidic channels using a key to set the valve to specific positions.*

Following blood collection, reagent volumes are precisely metered and mixed with assay components by pushing and pulling the liquid through the valve-controlled microfluidic channels. At the center of the cartridge is a sliding valve that functions as a manifold, controlling fluid pathways by sliding into different positions using a key. By


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completely filling the channels within the sliding valve before repositioning it, only the volume inside the valve is manipulated allowing for accurate metering to preserve reaction ratio essential for successful assay performance. The metered liquid is thoroughly mixed using a mixing chamber, and the resulting mixture is distributed into six individual chambers, each preloaded with dry virus-specific primers. Upon contact with the liquid, the primers rehydrate, initiating nucleic acid amplification via LAMP, where individually spatially distributed amplifications, termed 'blooms' within each compartment are recorded and analyzed using a smartphone camera and a customized app.

### Handheld System

Designed with field use and portability in mind, the detection system features a compact form (98 x 42 x 51 $mm^3$) and operates without the need for an external power source (**Figure 2**). To provide the thermal conditions required for LAMP, an inexpensive Peltier module is employed with a copper sheet affixed with thermal paste to enhance surface heat uniformity. A thermocouple probe embedded in the copper sheet enables a temperature feedback system, ensuring accurate thermal regulation under variable field conditions, a critical factor for the reliability of a LAMP-based diagnostic. Four high-powered LEDs, emitting at peak wavelength 485 nm, illuminate the reaction chamber at an oblique angle from the side (resembling dark-field illumination microscopy), allowing uniform lighting within the compact instrument while minimizing glare from the reflections off the transparent module adhesive tape. A macro lens, centrally positioned within the device, aligns with the smartphone camera via a slide-fit case, enabling image capture of the reaction chambers for downstream spatial-LAMP analysis. To ensure accurate fluorescence detection, a short-pass filter is placed in front of the LEDs, while a long-pass filter is positioned before the macro lens, isolating the fluorescent emission generated by the LAMP reaction. Fabricated using inexpensive, off-the-shelf electrical components and additive manufacturing (Formlabs), this compact, smartphone-assisted detection system, combined with its injection molded cartridge counterpart offers a cost-effective solution for POC molecular diagnostics.

### S-LAMP Image Analysis

A spatial LAMP (S-LAMP) image processing algorithm is used to accurately quantify and diagnose the LAMP assay results from the smartphone images. The S-LAMP analysis within this work builds off the framework set in the previous work, with several improvements for addressing the multiplex detection system[6]. Briefly, the smartphone takes images every 10 seconds, which are then converted to grey scale, using only the green RBG values. Each compartment region is analyzed to segment the region for LAMP 'blooms', regions of localized fluorescent amplification, rather than the global intensity within the compartment as in traditional fluorescence detection. Focusing on bloom detection allows for faster detection of fluorescence, improving time to result for this POC device.

## EXPERIMENTAL RESULTS

### Methods

Primer sequences were selected from literature and once primer solutions are created, dried on the cartridge. Zika primers targeted the NS1 gene[7]. Chikungunya primers targeted the E1 gene[8]. Dengue 1 targeted the NS2A gene, while Dengue 3 primers targeted the NS1 gene[9]. MS2 primers, which acted as the positive control, targeted the MS2g4 gene[10]. LAMP primers were first characterized on genomic RNA and then on virus strains obtained from BEI. Stocks were aliquoted then stored in -80°C until use. Aliquots were then diluted to desired concentration in TE buffer or whole blood. Human whole blood was obtained from BioIVT in 10 mL K2EDTA tubes which were stored at 4°C on an orbital shaker to prevent blood coagulation. All LAMP reactions occurred with the same master mix: 10x isothermal amplification buffer from New England Biolabs, 100mM $MgSO_4$, 5M betaine, 10mM dNTP mix, 8U $\mu L^{-1}$ Bst 2.0 Warmstart DNA polymerase, 15U $\mu L^{-1}$ Warmstart RTX reverse transcriptase, 25μM Evagreen dye, and 20 μg $\mu L^{-1}$ Bovine Serum Albumin.

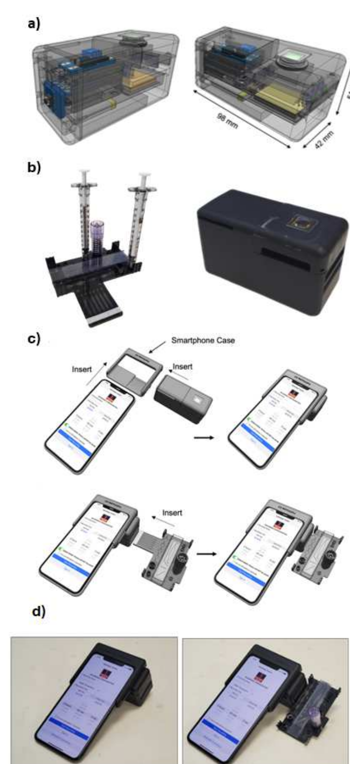


*__Figure 2__: A) Schematics of the handheld detection instrument B) Image of the assembled microfluidic cartridge and built detection instrument inside 3D printed body C) Demonstrated use of the system, with the smartphone inserted into case which is slide latched onto the detection instrument. Once the smartphone is attached, the injection molded instrument is inserted so the six compartments can be imaged. D) Images of the smartphone assisted detection instrument and injection molded cartridge assembled.*

### Limit of Detection

Once LAMP primers were verified on conventional thermocyclers in buffer and whole blood solutions, LAMP



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experiments were performed using the handheld system. In whole blood samples on-cartridge tests, we demonstrate detection limits of $1 \times 10^7$ , $2 \times 10^5$ , $6 \times 10^6$ PFU/mL for Chikungunya, Dengue 1 and Dengue 3 respectively as seen in **Figure 3**. Reduced amplification time corresponds to a higher virus concentration.

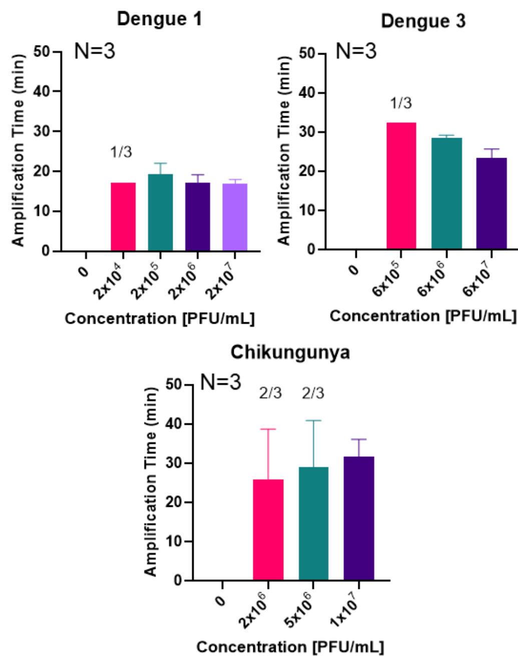


*Figure 3: Detection limits of the additional assays developed, determined through S-LAMP. Limit of detection for each assay was 1x10^7, 2x10^5, and 6x10^6 PFU/mL for Chikungunya, Dengue 1, and Dengue 3, respectively.*

### Cross Reactivity

Selectivity tests were performed using spiked whole blood solutions to confirm the system's ability to accurately determine positive/negative presence of target viruses without significant cross reactivity interference. High and medium virus concentrations were determined based on reported clinical patient ranges. The concentrations for high were $2 \times 10^6$ PFU/mL, $6 \times 10^6$ PFU/mL, $9 \times 10^6$ PFU/mL, $1 \times 10^6$ copies/mL for Dengue 1, Dengue 3, Chikungunya, and Zika respectively. MS2 as an internal standard was tested at the high concentration of $9 \times 10^7$ PFU/mL to confirm the phage presence would not create false amplification. Medium concentrations were 10-fold serial dilutions from high. Cross reactivity tests are shown in **Figure 4**.

### Multiplex Detection

The diagnostic capability of the system is demonstrated through the detection of multiple viruses in whole blood alongside the experimental controls (MS2 as positive, and primer free as negative). MS2 is spiked into blood for the multiplex detection assays at $1 \times 10^7$ PFU/mL. A standard layout for dried primers is developed shown in **Figure 5a**, which shows the raw image from one of the replicates showing Dengue 3 detection. The individual assays: Zika, Chikungunya, Dengue 1 and Dengue 3 are shown in **Figure 5b-e** at the highest clinical range. We also demonstrate the detection ability in comorbid infection situations with the detection of 3 viruses (Zika, Dengue 1 and Chikungunya) in 3 replicates shown in **Figure 5f**.

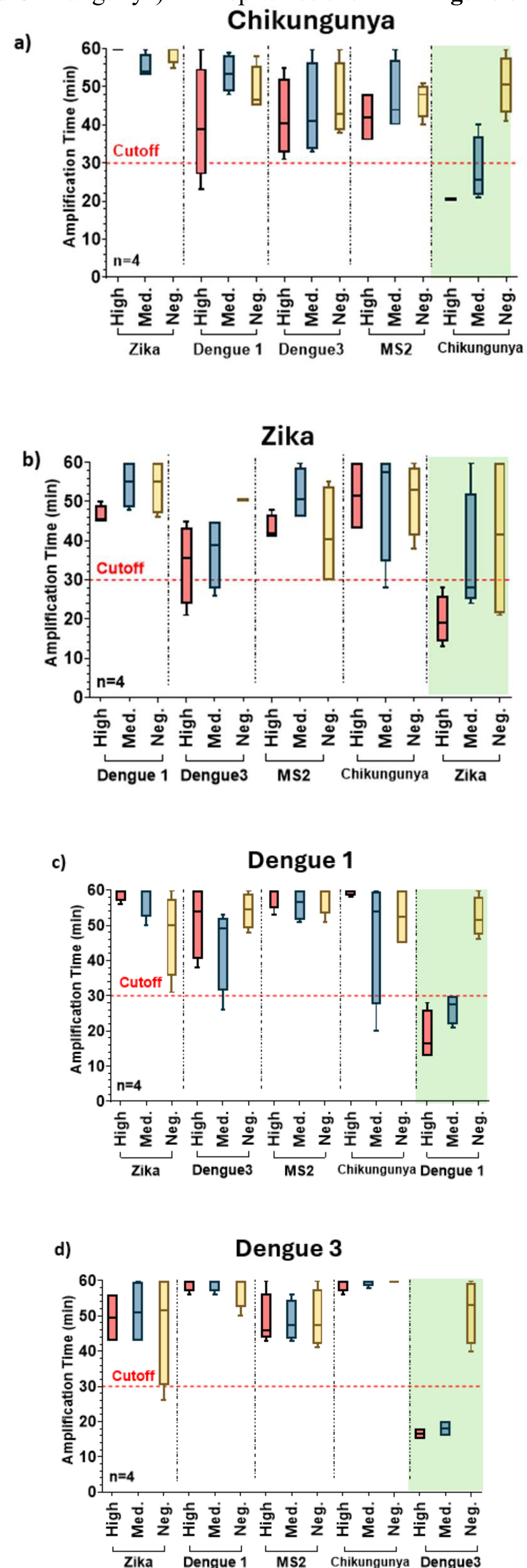


*Figure 4: Selectivity tests performed by spiking viruses into whole blood, demonstrating no significant cross reactivity between LAMP primers. A 30-minute cut off was set within the S-LAMP algorithm to detect true amplification and is indicated within the cross-reactivity data with the red line. Green background indicates a matching primer virus assay, while white background*



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*indicates mismatched primers and virus.*

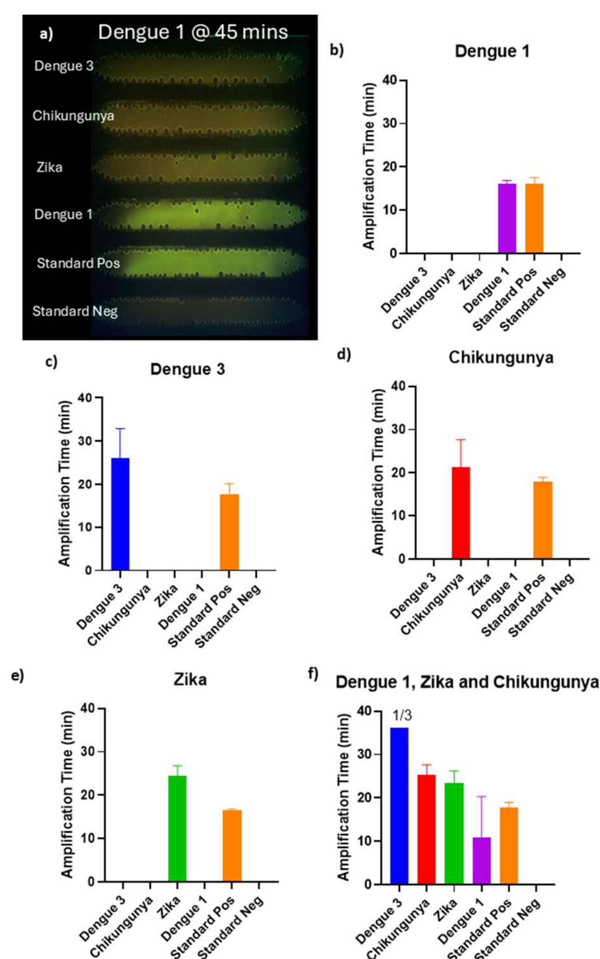


***Figure 5:*** *A) Raw image from the detection instrument showing the six fluid compartments and corresponding LAMP primer layouts for the multiplexing assays. B-E) Measured amplification times for the multiplex arrays within this set up demonstrating no cross reactivity. F) Demonstrated multiplex amplification times for detection of 3 viruses. All assays have N=3.*

# CONCLUSION

We have demonstrated a field ready detection system by developing a microfluidic processor that eliminates pipette need as well as does all the sample handling from a finger-prick of blood (35 µL). This detection system is also completely portable as it does not rely on external power sources and is handheld sized. This system demonstrates multiplexed capabilities for detecting Zika, Chikungunya and two serotypes of Dengue (1 and 3) in under 30 minutes from a single whole blood sample.

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# REFERENCES

[1] P. A. H. Organization, *Guidelines for the Clinical Diagnosis and Treatment of Dengue, Chikungunya, and Zika*, 2021.

[2] Z. J. Madewell, “Arboviruses and Their Vectors,” *South Med J,* vol. 113, no. 10, pp. 520-523, Oct, 2020.

[3] FDA. "FDA Approves First Vaccine to Prevent Disease Caused by Chikungunya Virus," https://www.fda.gov/news-events/press-announcements/fda-approves-first-vaccine-prevent-disease-caused-chikungunya-virus.

[4] S. Kharwadkar, and N. Herath, “Clinical manifestations of dengue, Zika and chikungunya in the Pacific Islands: A systematic review and meta-analysis,” *Reviews in Medical Virology,* vol. 34, no. 2, pp. e2521, 2024.

[5] K. J. Land, D. I. Boeras, X.-S. Chen, A. R. Ramsay, and R. W. Peeling, “REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes,” *Nature Microbiology,* vol. 4, no. 1, pp. 46-54, 2019/01/01, 2019.

[6] A. M. Jankelow, H. Lee, W. Wang, T.-H. Hoang, A. Bacon, F. Sun, S. Chae, V. Kindratenko, K. Koprowski, R. A. Stavins, D. D. Ceriani, Z. W. Engelder, W. P. King, M. N. Do, R. Bashir, E. Valera, and B. T. Cunningham, “Smartphone clip-on instrument and microfluidic processor for rapid sample-to-answer detection of Zika virus in whole blood using spatial RT-LAMP,” *Analyst,* vol. 147, no. 17, pp. 3838-3853, 2022.

[7] M. Sabalza, R. Yasmin, C. A. Barber, T. Castro, D. Malamud, B. J. Kim, H. Zhu, R. A. Montagna, and W. R. Abrams, “Detection of Zika virus using reverse-transcription LAMP coupled with reverse dot blot analysis in saliva,” *PLOS ONE,* vol. 13, no. 2, pp. e0192398, 2018.

[8] M. M. Parida, S. R. Santhosh, P. K. Dash, N. K. Tripathi, V. Lakshmi, N. Mamidi, A. Shrivastva, N. Gupta, P. Saxena, J. P. Babu, P. V. L. Rao, and K. Morita, “Rapid and Real-Time Detection of Chikungunya Virus by Reverse Transcription Loop-Mediated Isothermal Amplification Assay,” *Journal of Clinical Microbiology,* vol. 45, no. 2, pp. 351-357, 2007.

[9] S.-f. Hu, M. Li, L.-l. Zhong, S.-m. Lu, Z.-x. Liu, J.-y. Pu, J.-s. Wen, and X. Huang, “Development of reverse-transcription loop-mediated isothermal amplification assay for rapid detection and differentiation of dengue virus serotypes 1–4,” *BMC Microbiology,* vol. 15, no. 1, pp. 265, 2015/11/14, 2015.

[10] Y. Chander, J. Koelbl, J. Puckett, M. J. Moser, A. J. Klingele, M. R. Liles, A. Carrias, D. A. Mead, and T. W. Schoenfeld, “A novel thermostable polymerase for RNA and DNA loop-mediated isothermal amplification (LAMP),” *Frontiers in Microbiology,* vol. 5, 2014-August-01, 2014.

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