# Supporting Information

# Mayaro Virus Detection by Integrating Sample Preparation with Isothermal Amplification in Portable Devices

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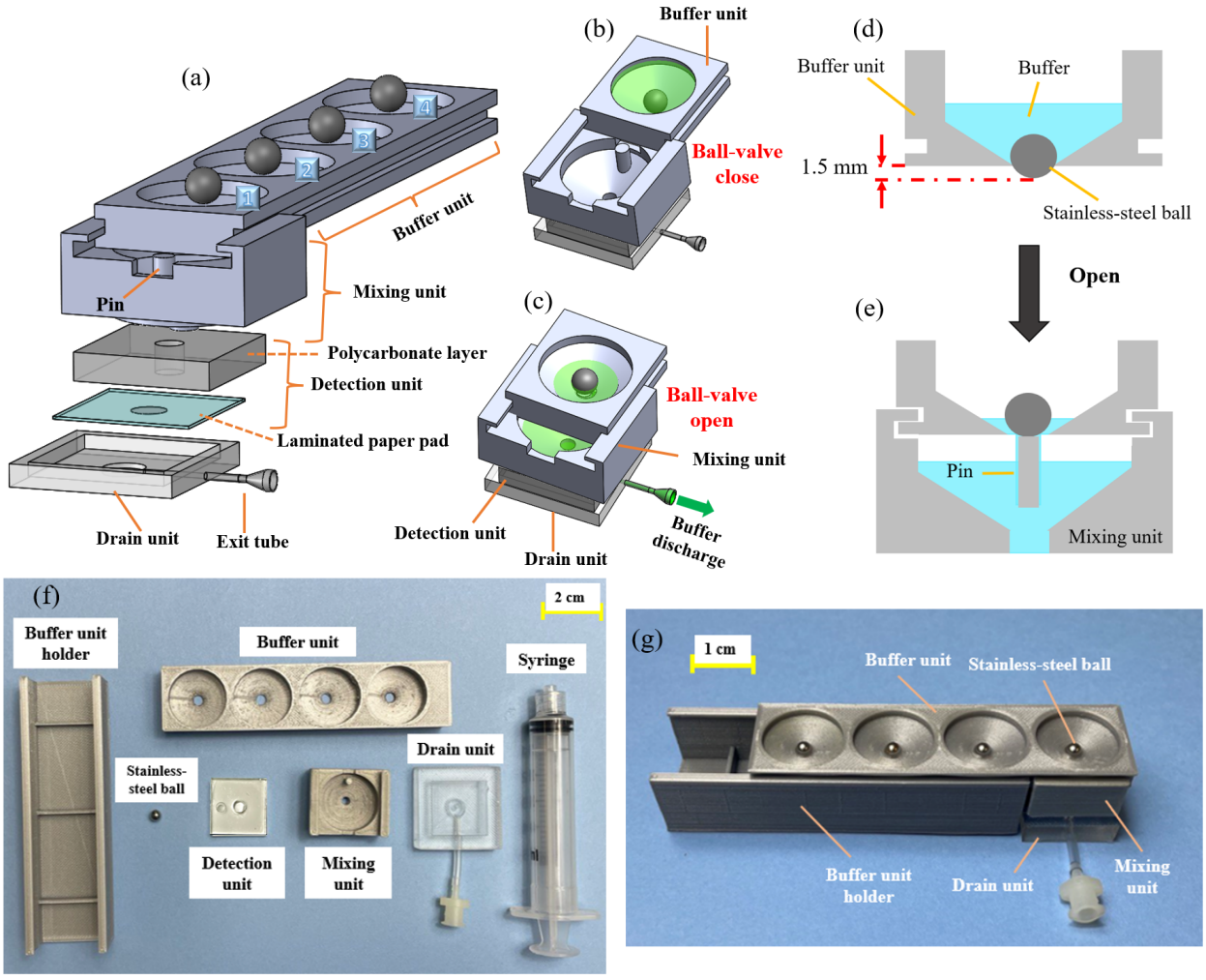


**Figure S1**. (a) Exploded view of the SPD (sample preparation device) consisting of a buffer unit, a mixing unit, a detection unit, and a drain unit. The buffer unit comprises 4 reservoirs for the storage of a lysis buffer (reservoir 1), a binding buffer (reservoir 2), and 2 washing buffers (reservoirs 3 and 4). In each funnel-shaped reservoir, one stainless-steel ball is placed at the bottom that functions as a valve, preventing buffers from flowing down until it is desired (the balls are above the reservoirs for visualization). To ensure that the ball remains secure to prevent any undesired movement or leakage during transportation, wax is employed to fix the ball in place. The process starts with melting a piece of wax (Akrowax™ 130, Akron, OH, USA) in a small beaker, immersing the ball into the melted wax, followed by placing the ball, coated with a thin layer of wax, in the reservoir where the wax solidifies, forming a bond that can be broken when needed. The mixing unit contains a well and a pin, and it slides along the buffer unit through the sliding tracks on both sides of the buffer unit. When the pin in the mixing unit is aligned with the ball, it breaks the wax bond and lifts the ball to open the valve, allowing the solution to flow down through the mixing unit and into the detection unit. The paper pad in the detection unit (2 cm x 2 cm) is used to collect RNA for subsequent RT-LAMP. The drain unit is integrated to the detection unit and it is designed to speed up the reagent discharge by connecting a syringe to the exit tube. (b) and (c) Valve actuation enabled by sliding the mixing unit (b) to align the pin with the ball, causing the ball to be lifted up and reagents discharged into the mixing unit (c). (d) and (e) are the

cross-sectional view of (b) and (c). (f) Photograph of all components in SPD. The buffer and mixing units were 3D-printed using polylactic acid (PLA). The detection unit was made from a paper pad attached to a polycarbonate well layer. The drain unit was made by casting PDMS into a 3D printed mold and curing at 100 ºC for 1 hour. The mold consisted of a reverse pattern of the drain unit. (g) Photograph of an assembled SPD, in which the mixing unit slides along with the buffer unit, lifts balls in the reservoirs, sequentially discharging the buffers stored in the reservoirs. The detection unit is attached to the bottom of the mixing unit and placed in the drain unit's chamber. The exit tube of the drain unit is connected to a syringe as shown in the Electronic Supplementary Material (Video 2).

**Table S1.** Comparison between VLEAD and SPD.

| | **VLEAD** | **SPD** |
|---|---|---|
| **Discharge mechanism** | Wicking effects or capillary force | Syringe-induced vacuum |
| **Suitable samples** | Low viscous samples such as saliva and urine | Both low and high viscous samples such as saliva, urine, and blood |
| **Discharge time using blood samples** | ~3 hours | 2 minutes |

**Table S2.** Components and their volumes used in the 25-µL RT-LAMP mixture.

| **Components** | **dNTP** | **ISO** | **PMX** | **MgSO4** | **Bst** | **RTx** | **dUTP** | **UDG** | **Water** |
|---|---|---|---|---|---|---|---|---|---|
| **Volume (µL)** | 3.5 | 2.5 | 2.5 | 1.5 | 1 | 0.5 | 0.5 | 0.5 | 12.5 |

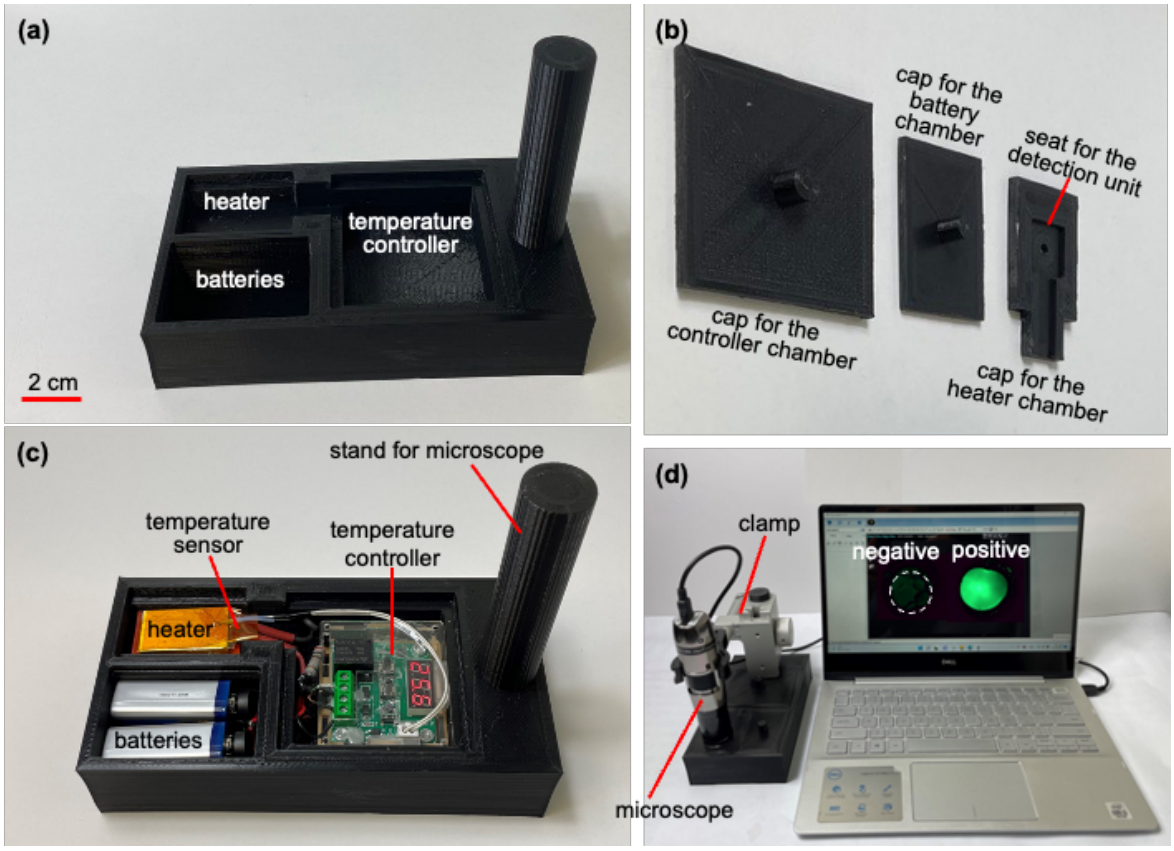


**Figure S2**. Photographs of the real-time amplification device (RAD). (a) 3D-printed stand with a base consisting of three chambers for a heater, batteries, and an integrated electronic circuit as a temperature controller. Black polylactic acid (PLA) was used as print material. (b) 3D-printed caps for three chambers in (a). A cavity is created in the cap of the heater chamber and the detection unit in Figure S1 can fit there properly. (c) The same stand in (a) with all electronic components in the place, but without chamber caps. (d) Assembled RAD connected with a computer. On the computer screen was an image of two wells in the detection unit: one well for the negative control which is dark (indicated by dashed lines) while the other well for a sample that had a positive signal.

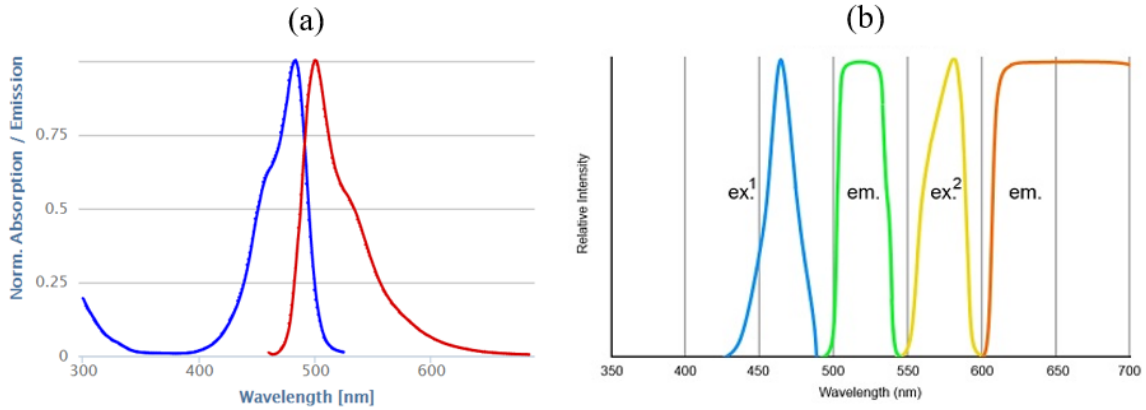

**Figure S3**. Spectrum charts of (a) dye SYTO9 and (b) the flashlight-shaped microscope, both of which were provided by the corresponding manufacturers.

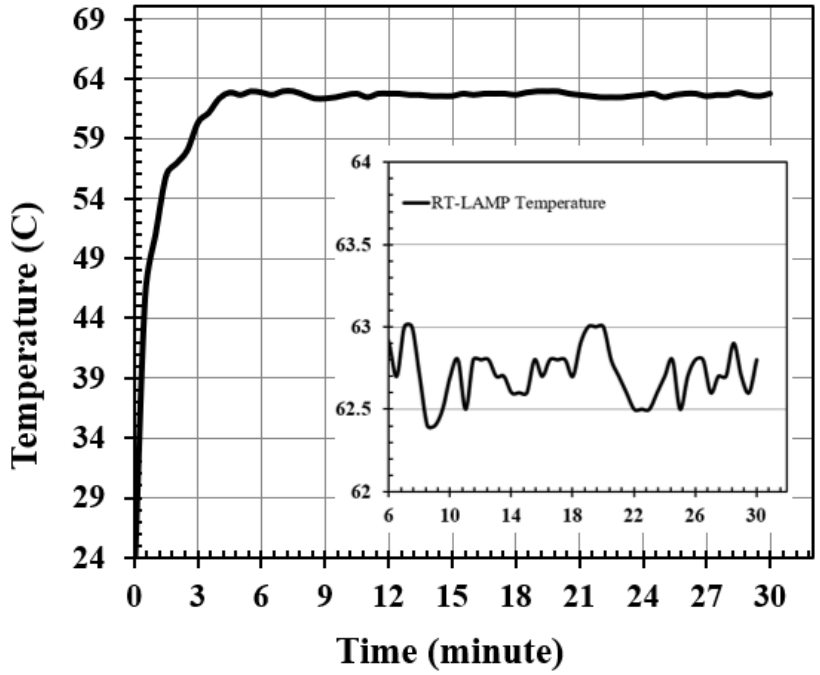


**Figure S4**. Temperature profile of the RAD in Figure S2. The temperature was measured using a T-type thermocouple that was embedded in the rRT-LAMP reaction mixture. The temperature reached 62.5 $^{o}$C in 5-min., thus the heater was turned on to reach the temperature equilibrium at least 5 min. before the rRT-LAMP. The inset shows the temperature fluctuation in rRT-LAMP reaction mixture while assay was being performed; the temperature is within the range of 60-65 $^{o}$C recommended for rRT-LAMP.


**Figure S5**. Photographs of a commercially available coffee mug used as a water bath for isothermal amplification in the format of endpoint detection. The mug is powered by a rechargeable battery and controlled using a smartphone App. The subsequent detection of the amplicons of RT-LAMP is achieved by adding 0.5 µL of 10,000X concentrate SYBR green dye at the end and the color change can be observed by the naked eye if MAYV is present. To enhance visualization, a blue LED flashlight is used to illuminate the reaction mixture, and fluorescence signal would be observed. A no-template control is included, as a negative control, in each test. The test results, i.e., the image of detection units, are captured using a smartphone camera and the presence/absence of the amplicons after RT-LAMP is confirmed by gel electrophoresis.

**Primer set #1**

241 CGAAAAAGAC ACACTCATCC TGGATATCGG CAGTGCGCCT GCCAGGAGAA TGATGTCTGA
F3 F2

301 GCACACGTAC CATTGTGTGT GCCCAATGCG CAGCGCTGAG GACCCAGAGC GTCTGCTGTA
FLP F1C

361 TTATGCCAGG AAGTTAGCCA AGGCATCAGG TGAAGTCGTT GACAGAAATA TTGCAGCGAA
B2 BLP

421 GATAGACGAC CTGCAGTCAG TGATGGCGAC CCCGGACAAT GAGTCACGGA CATTTTGCCT
B1C B3

**Primer set #2**

421 GATAGACGAC CTGCAGTCAG TGATGGCGAC CCCGGACAAT GAGTCACGGA CATTTTGCCT
F3

481 TCACACAGAT CAGACATGCA GGACTCCAGC TGAGGTGGCA GTCTATCAGG ATGTCTATGC
F2 FLP

541 AGTTCACGCA CCGACTTCTT TGTACTTCCA GGCAATGAAA GGAGTACGCA CAGCGTACTG
F1C

601 GATTGGGTTC GACACTACCC CATTCATGTT CGATACAATG GCCGGGGCTT ATCCAACATA
BLP B1C

661 TGCAACCAAC TGGGCCGACG AACAGGTGTT GAAAGCCAGG AACATAGGAC TGTGCTCCGC
B3

**Figure S6**. Sequences and location of two sets of rRT-LAMP primers designed for detecting Mayaro virus (MAYV). The genomic sequence of MAYV can be accessed from GenBank at the National Center for Biotechnology Information (NCBI) with an accession #DQ001069.1

**Table S3.** Primer concentrations used in RT-LAMP

| Primer | NEB (µM) | 1/2NEB (µM) |
|---|---|---|
| **F3 and B3** | 2 | 1 |
| **FIP and BIP** | 16 | 8 |
| **FLP and BLP** | 4 | 2 |

NEB: New England Biolabs, the kit manufacturer; 1/2NEB: at the half of the recommended values

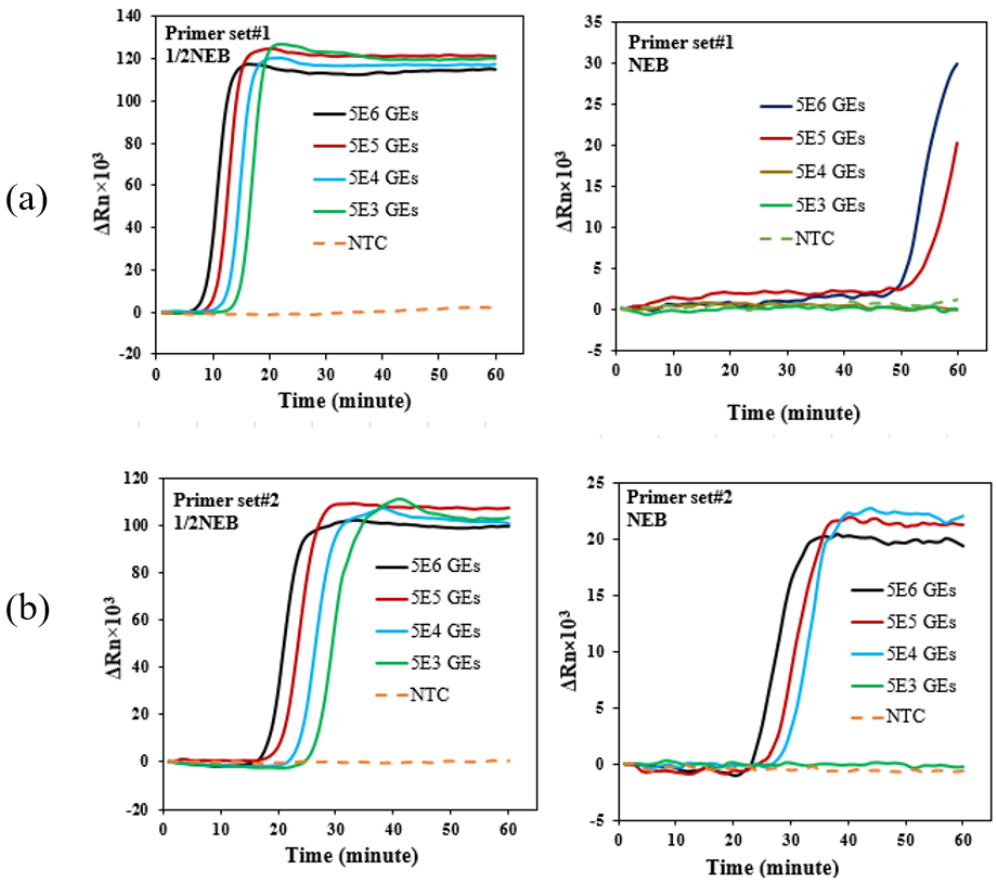


**Figure S7**. rRT-LAMP of MAYV RNA using (a) primer set #1 or (b) primer set #2 using primer concentrations at the values recommended by NEB or at 1/2NEB. The amount of MAYV ranges from $5\times10^6$ to $5\times10^3$ genome equivalents (GEs). Each curve is an average of three replicates. The y-axis ($\Delta R_n$) is fluorescence signal obtained by the real-time PCR instrument. NTC stands for no-template control.

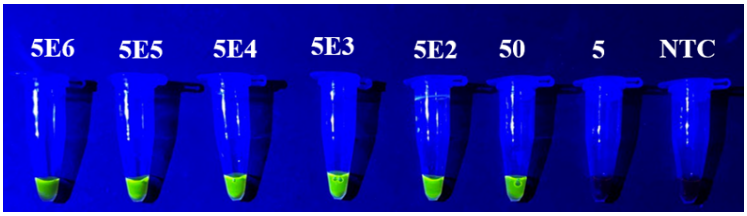


**Figure S8**. Endpoint RT-LAMP assay for detection of $5\times10^6$ to 5 GEs/ µL of MAYV RNA. The endpoint detection was carried out after 30 min RT-LAMP reactions. NTC stands for no-template control.

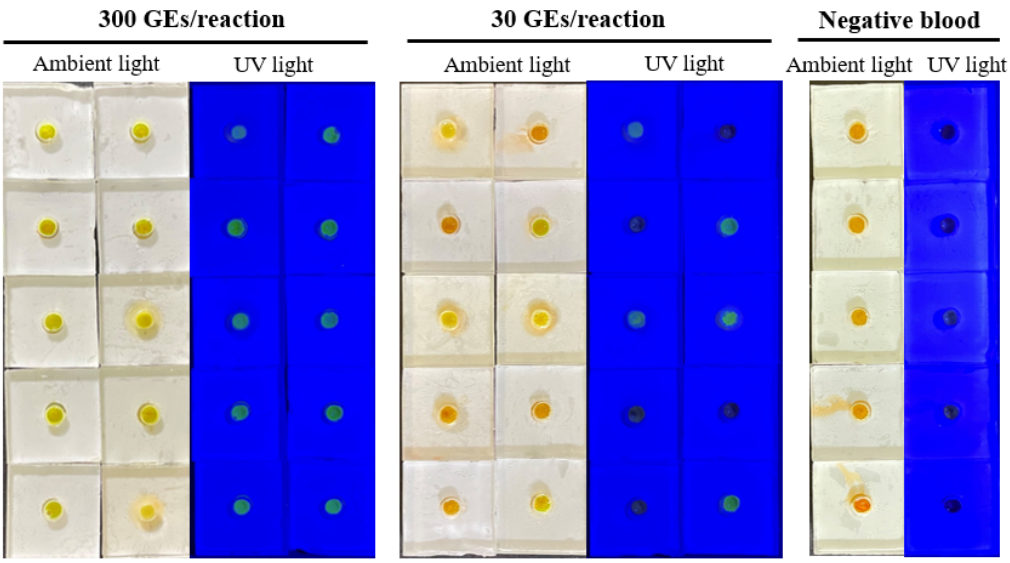


**Figure S9**. Detection of MAYV in whole blood using SPD. Pictures of the detection units under ambient light and blue LED after RT-LAMP assay of whole blood samples with 6 and 0.6 GEs/μL of MAYV and blood samples without MAYV as negative blood. At each test, 50 μL of the blood sample was used.

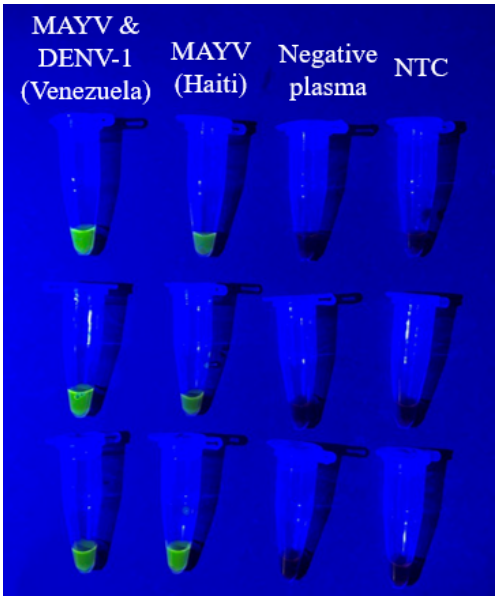


**Figure S10**. Results of the RT-LAMP assay designed for detecting MAYV when a set of clinical samples were used. These samples include one from a Haitian patient infected with MAYV, one from a Venezuelan patient infected with both DENV-1 and MAYV, and one blood plasma from a Haitian free of viruses. A PCR machine was used in these experiments, and the incubation time was 30 min., followed by the endpoint detection.

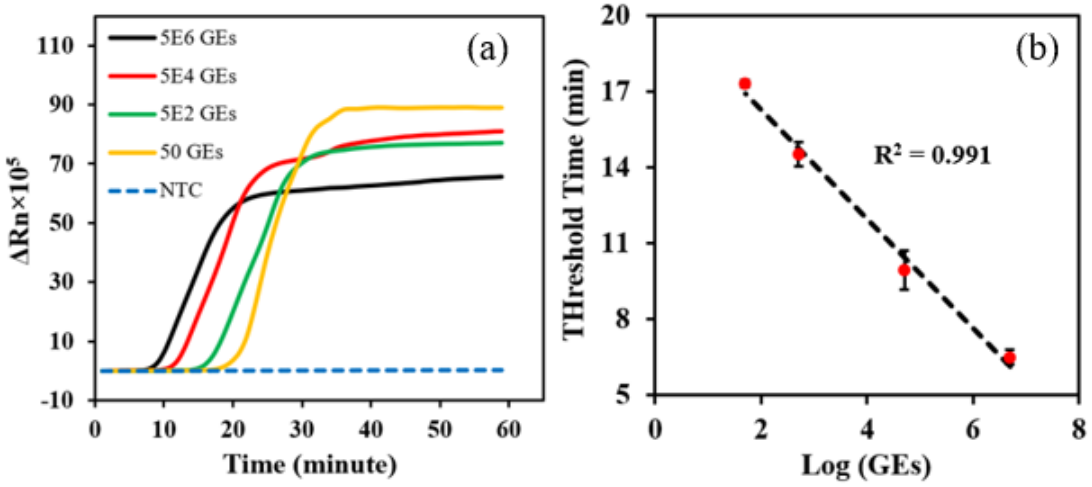


**Figure S11**. (a) Real-time amplification curves for MAYV RNA at $5x10^6$ to 50 GEs using a real-time PCR machine. The NTC stands for no-template control. (b) A calibration curve between the threshold time and the amount of MAYV. The threshold time was provided by the commercial PCR machine.

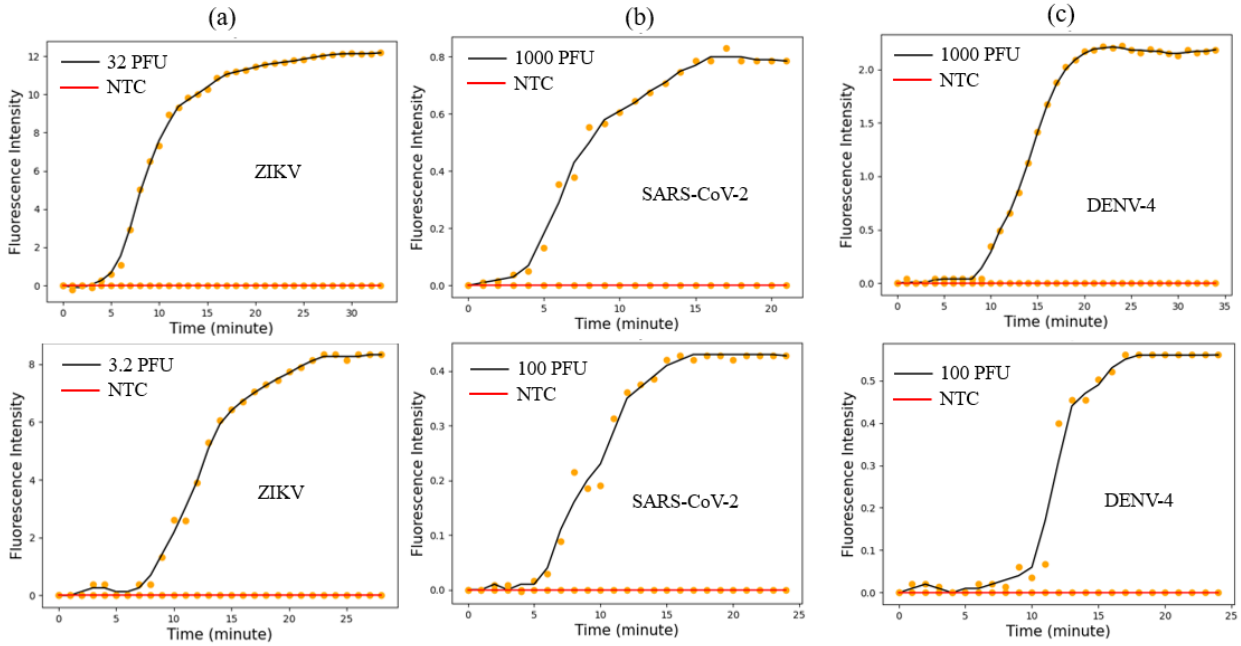


**Figure S12.** Amplification curves generated from RAD in Figure 1 in the main text. Three different viruses of (a) ZIKV [1], (b) SARS-CoV-2 [2], and (c) DENV-4 [3] were used. one replicate was carried out for each concentration. The orange dots are raw data, and the lines are fitted curves. The fit curve was generated by averaging three data points. 1/2 NEB concentration of the primers were used to prepare the RT-LAMP reaction mixture for each virus.

## References

[1] X. Jiang, J.C. Loeb, C. Manzanas, J.A. Lednicky, Z.H. Fan, Valve-Enabled Sample Preparation and RNA Amplification in a Coffee Mug for Zika Virus Detection, Angew Chem Int Ed Engl, 57(2018) 17211-4.

[2] C. Manzanas, M.M. Alam, J.C. Loeb, J.A. Lednicky, C.Y. Wu, Z.H. Fan, A Valve-Enabled Sample Preparation Device with Isothermal Amplification for Multiplexed Virus Detection at the Point-of-Care, ACS Sens, 6(2021) 4176-84.

[3] B. Lopez-Jimena, M. Bekaert, M. Bakheit, S. Frischmann, P. Patel, E. Simon-Loriere, et al., Development and validation of four one-step real-time RT-LAMP assays for specific detection of each dengue virus serotype, PLoS Negl Trop Dis, 12(2018) e0006381.