Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Zika Virus Lateral Flow Assays using Reverse Transcription-Loop-mediated Isothermal Amplification

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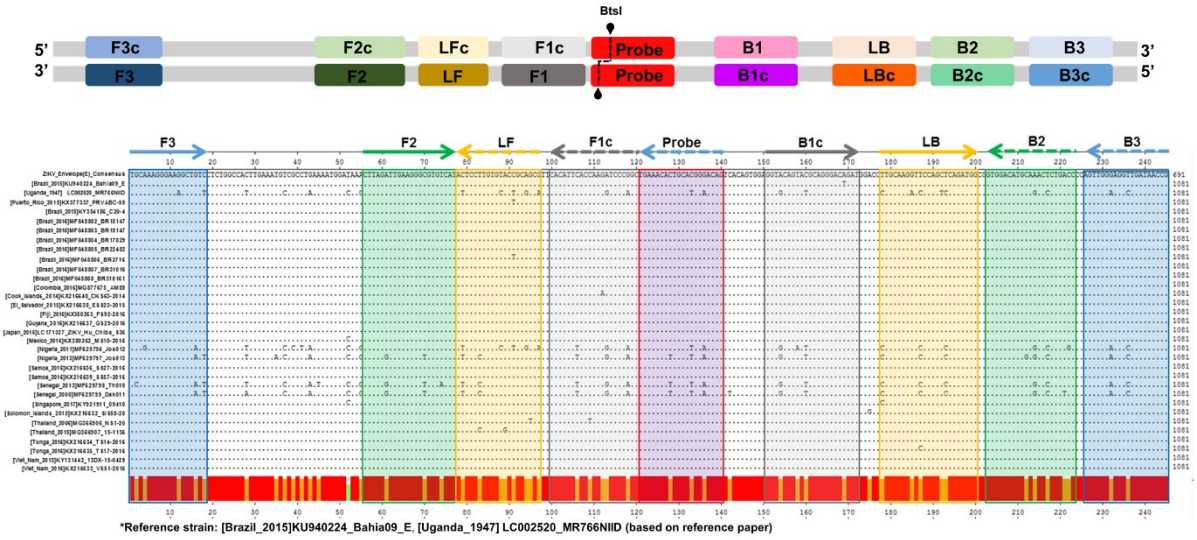

Figure S1. Design of the ZIKV LAMP primers and ZIKV target DNA fragment. The target gene codes for the Envelope protein-coding genes of the ZIKV strains. The different colors correspond to the different primer sequences of the LAMP primer set presented in Table 1. Restriction site of the enzyme (BtsI) used to confirm the ZIKV LAMP products is shown in red-probe boxes.

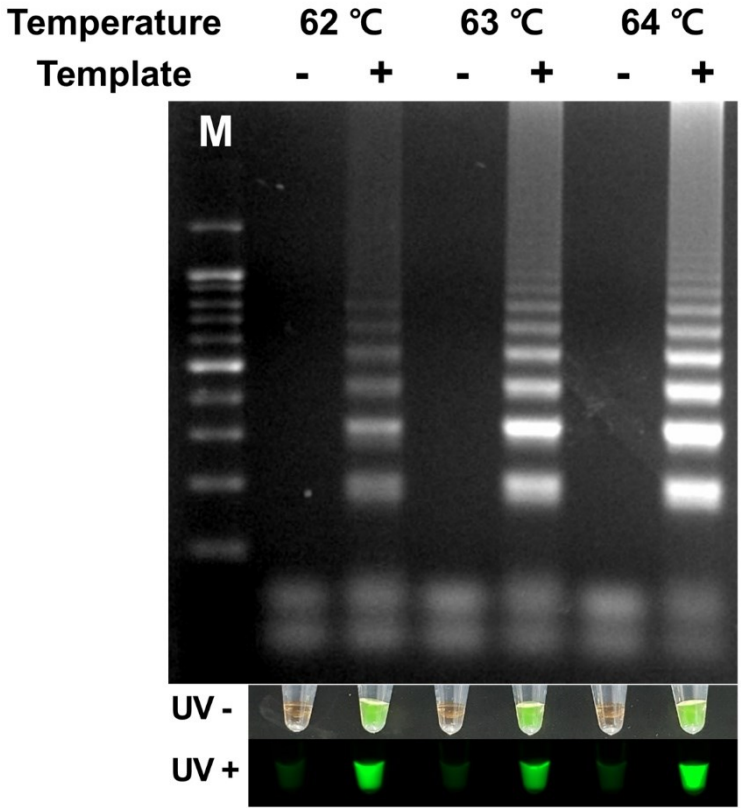

Figure S2. ZIKV RT-LAMP and visible detection amplification of the LAMP products. The results were detected via 1% agarose gel electrophoresis. Through a visual analysis, the true-positive ZIKV RT-LAMP results were recognized under natural light (UV OFF (-)) or UV irradiation (UV ON (+)) using SYBR Green I. Colorimetric changes from orange to green and fluorescence indicate a target-specific ZIKV RT-LAMP reaction.

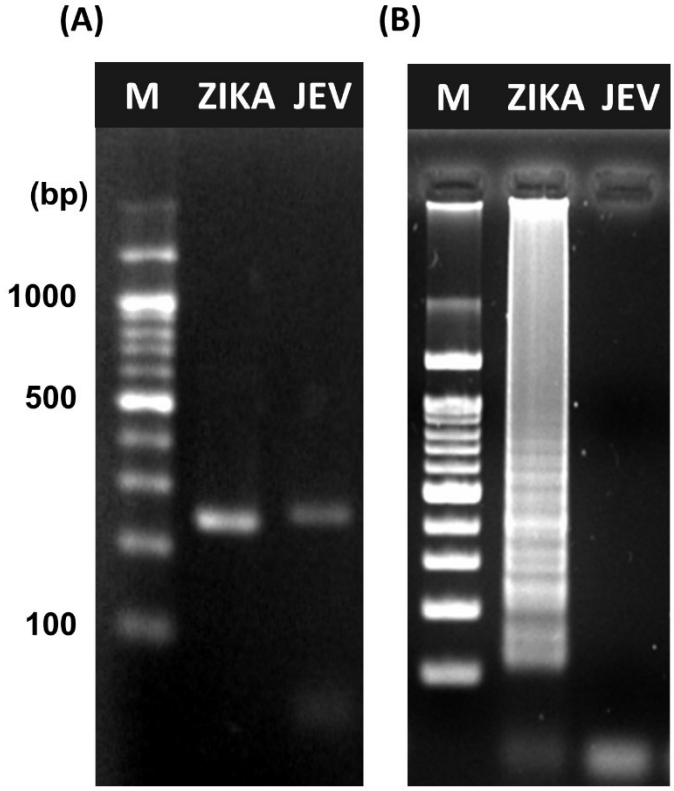

Figure S3. Virus-specific PCR and ZIKV RT-LAMP. (A) Virus-specific PCR. The following primers for JEV were synthesized by Cosmogenetech Co., Ltd. JEV: F primer 5′-GCACAAAGCTGGAAGCAC-3′; R primer 5′-TCGTGCGTTGACACCCAT-3′. ZIKV: Z_F3 primer 5′-TGCAAAGGGAAGGCTGTC-3′; Z_B3 primer 5′-CGGYYAYCAACCTCCCAACT-3′ (B) Both the ZIKV and JEV samples were assayed using ZIKV RT-LAMP primers (see Table 1). Conventional RT-LAMP had successful amplifications, as confirmed via 1% agarose gel electrophoresis.

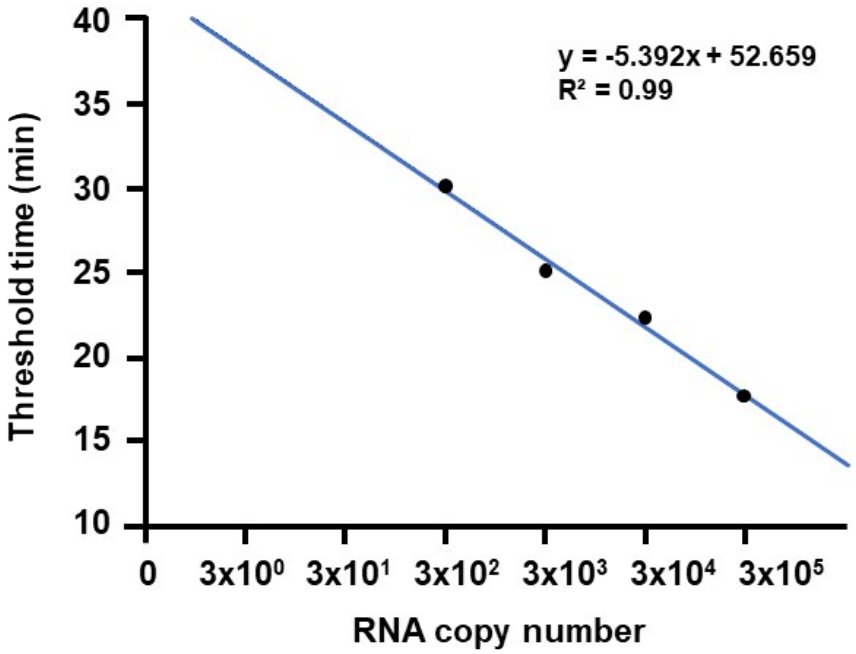

Figure S4. Calibration curve for ZIKV rtRT-PCR. Calibration curve was generated using known concentration of 10-fold serially diluted ZIKA RNA copy number and the threshold time value.

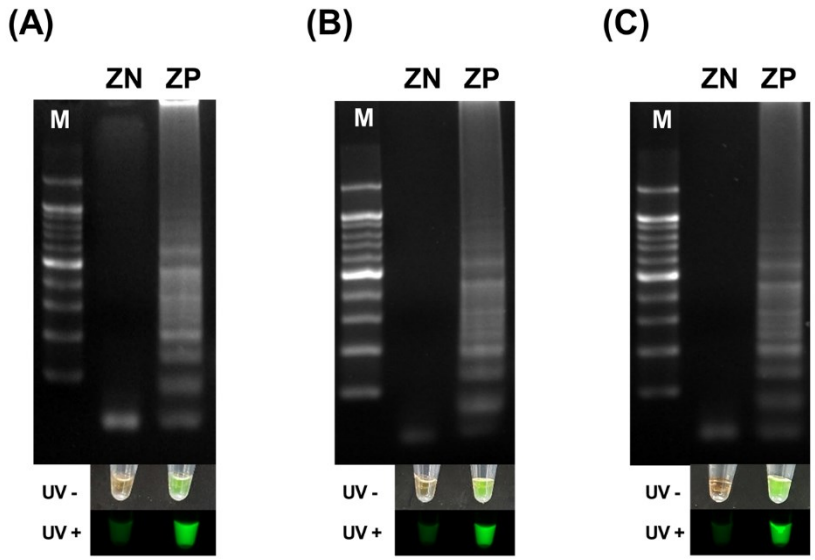

Figure S5. ZIKV RT-LAMP in complex samples. (A) PBS, (B) urine, and (C) serum outputs. The results were detected via 1% agarose gel electrophoresis. Through a visual analysis, the true-positive ZIKV RT-LAMP results could be recognized under natural light (UV OFF (-)) or UV irradiation (UV ON (+)) using SYBR Green I. Colorimetric changes from orange to green and fluorescence indicate a target-specific ZIKV RT-LAMP reaction. ZN, ZIKV Negative; ZP, ZIKV Positive.

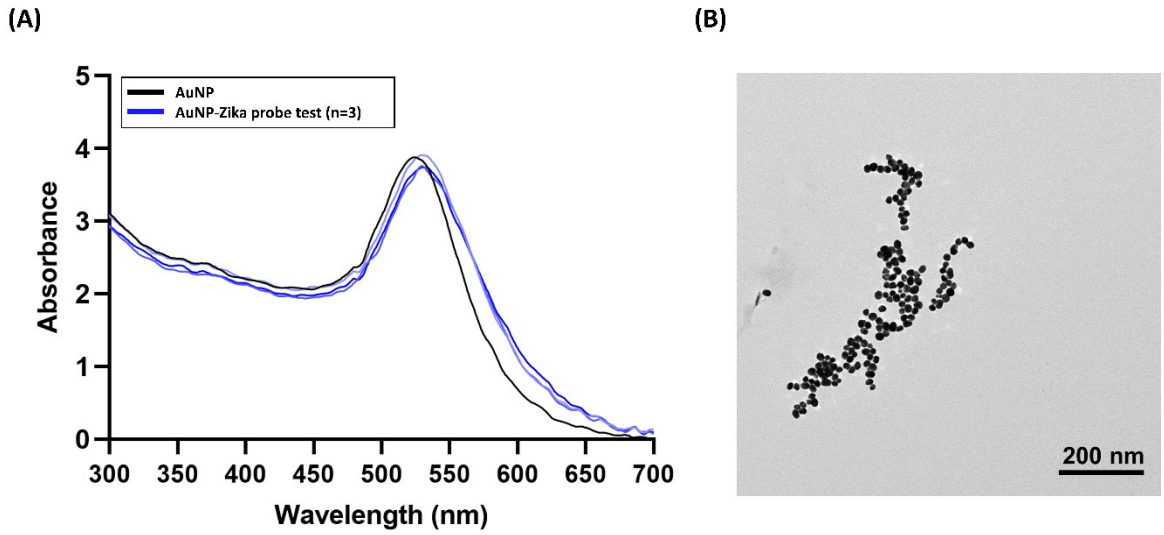

Figure S6. Stability of the AuNP:ZIKV probe. (A) Comparison of the typical absorption spectrum of AuNP to that of the AuNP:polyA$_{10}$-ZIKV probe. (B) TEM image of AuNP-Zika probe. The size and morphology of the AuNPs were observed using a Libra 120 transmission electron microscopy (TEM; Carl Zeiss, Oberkochen, Germany).

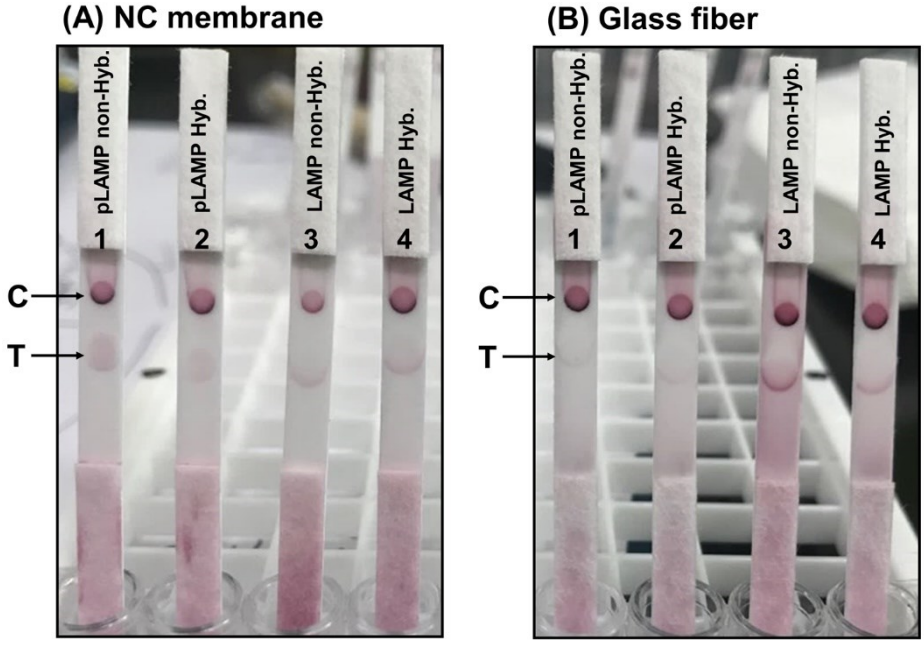

Figure S7. Hybridization between the LAMP amplicons and the AuNP:ZIKV probe. Lateral flow assay (LFA) detection results with/without hybridization (62 ℃, 5 min). After the LAMP amplicon was purified, 500 ng of the purified product was mixed with the AuNP:ZIKV probe and added into the LF strip to exclude the interference induced by the LAMP buffer in LFA detection. Purification of the LAMP amplicon was performed according to the manual provided in the PCR purification kit (Cosmogenetech, Korea). 1: LAMP purification, non-hybridization (pLAMP non-Hyb.); 2: LAMP purification, hybridization (pLAMP Hyb.); 3: LAMP, non-hybridization (LAMP non-Hyb.); and 4: LAMP, hybridization (LAMP Hyb.) (A) on the nitrocellulose membrane and (B) Glass fiber. The AuNP:polyA$_{10}$-ZIKV probe had a high background in the glass fiber membrane pad; however, background in the nitrocellulose membrane appeared very low owing to absorption by the absorption pad.