Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

Molecular-level dengue fever diagnostic devices made out of paper

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We have transcribed reversely and then amplified dengue virus serotype-2 RNA via using RT-LAMP for one hour at 63°C with concentrations of virus particles ranging from 6×10^{5} to 60 PFU/mL (**Supplementary Figure S1**). In these three panels, lane M was the 100-b.p. DNA marker; from lane 1 to 5, they were RT-LAMP results with the concentrations of virus particles from 6×10^{5} to 6×10^{-3} PFU/mL (hundred-fold dilution), respectively, and lane 6 was the negative control without the dengue virus serotype-2 RNA. These results showed that the sensitivity of RT-LAMP that we used in this study was enough to detect the virus particles with the concentration of about 60 PFU/mL in the buffer system; in this study, the virus particle concentration that we used for RT-LAMP reaction was about 6000 PFU/mL. Comparisons to the DNA electrophoresis results, while performing RT-LAMP reactions at different reaction durations (i.e., 45, 60 and 75 minutes in this study), we would think that the reaction duration for 45 minutes was not sufficient to complete the entire RT-LAMP reaction, in particular, specific to dengue virus serotype-2 RNA. Through the fluorescence intensity analysis of DNA electrophoresis gel (the amplified products, double-stranded DNA), we found that the fluorescence intensities of lane 1, 2 and 3 were about the same, and the ratios between lane 1 to lane M (100-b.p. DNA marker), and lane 2 to lane M (100-b.p. DNA marker) in terms of the concentration (i.e., the fluorescence intensity) of amplified products were about the same as well while performing RT-LAMP for either 60 or 75 minutes at 63°C. These results indicate that we could obtain the maximum concentration of the amplified products (double-stranded DNA) while carrying out RT-LAMP for 60 minutes at 63°C, and the concentration of these amplified products would not be increased significantly by increasing the RT-LAMP reaction duration from 60 to 75 minutes, even though the initial concentration of virus particles was different, consistently being congruent with the previous studies[1]. We also found that the lane 3 (75-minute RT-LAMP reaction) showed a lower intensity than lane 1 and 2 in the right panel, but the fluorescence intensity of the lane 3 (in the right panel) was not consistent with the lane 3 (60-minute RT-LAMP reaction) in the middle panel. Both lane 1 and 2 (75-minute RT-LAMP reaction) in the right panel exhibited no significant difference in the fluorescence intensity (with various concentrations), because the initial concentration of these two RT-LAMP reactions was much higher than the RT-LAMP reaction threshold but the initial RNA concentration to start the RT-LAMP reaction (displayed in lane 3, in the right panel) was close to the RT-LAMP reaction threshold, showing a lower fluorescence intensity than lane 1 and 2, in the right panel[2].

Figure S1. DNA electrophoresis results of dengue virus RT-LAMP assays after 45-, 60- and 75-minute reaction. Lane M, 100-b.p. DNA ladder; lane 1, 6×10^{5} PFU/mL; lane 2, 6×10^{3} PFU/mL; lane 3, 60 PFU/mL; lane 4, 6×10^{-1} PFU/mL; lane 5, 6×10^{-3} PFU/mL; lane 6, negative control without the dengue virus serotype-2 RNA replaced by ddH_2O.

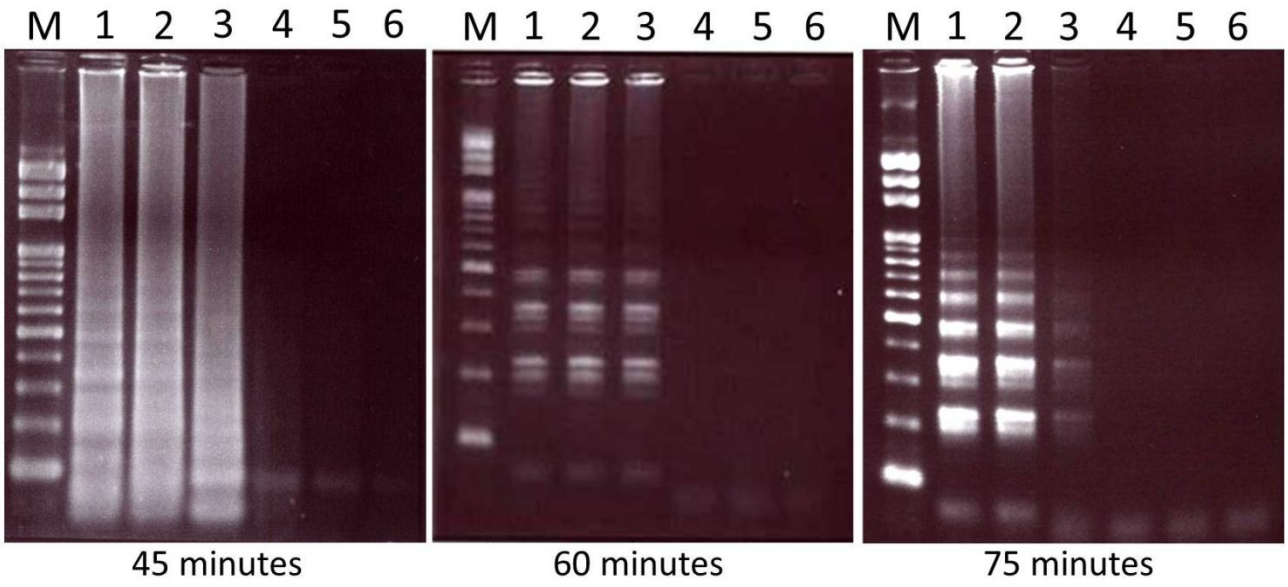
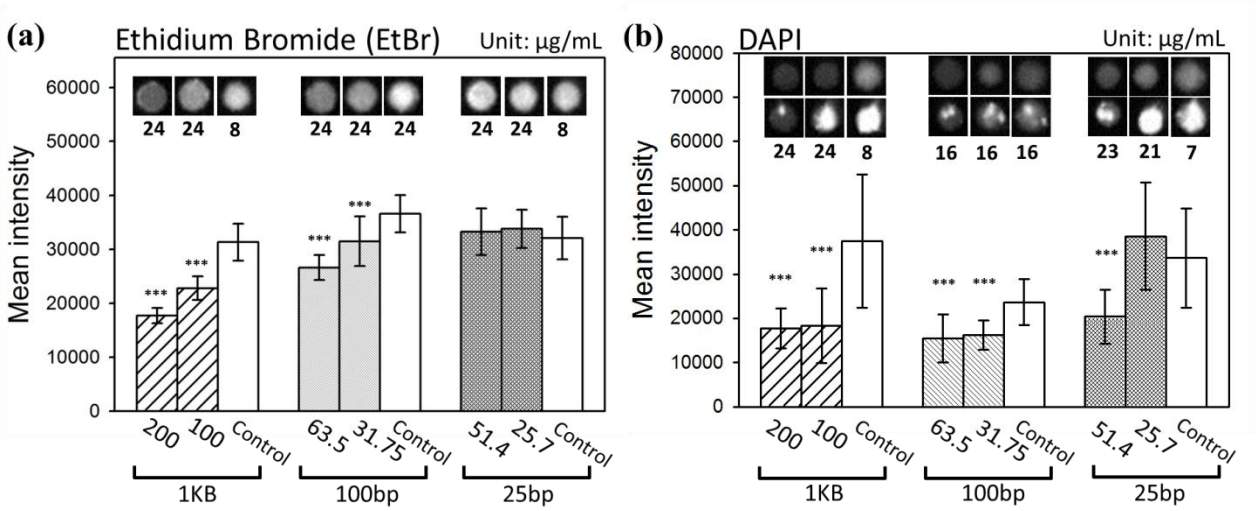
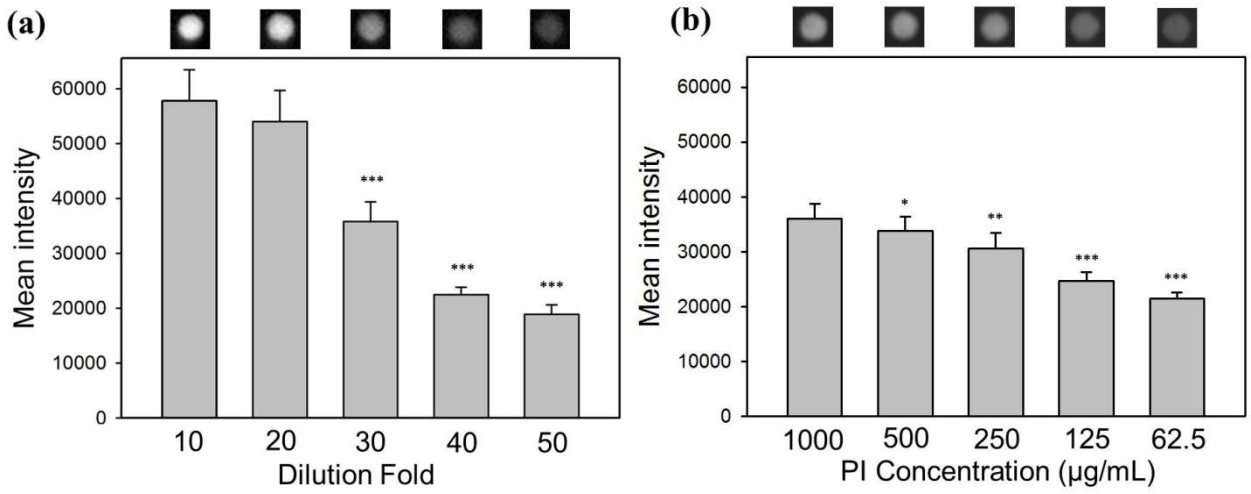


Figure S2. Fluorescence intensity profiles of using nucleic acid probes to label various types of DNA ladders with different concentrations in paper-based test zones. The x- and y-axes represent the average concentration while using different DNA ladders (i.e., different ladder sizes) and the fluorescence intensity, respectively. The upper inset images in both (a) and (b) showed the change of fluorescence intensity profiles with different DNA ladder concentrations. (a) Ethidium bromide showed an intensity increase but (b) DAPI did not, when the concentration of DNA ladders decreased. We also found that DAPI molecules aggregated in paper-based test zones. Each *N* number was presented under each inset image. *p < 0.05; **p < 0.01; ***p < 0.001(Student's t-test), indicating statistically significant differences compared with the control group.



We attempted to optimize the amount of both U-safe and PI fluorescence probes that we used in paper-based test zones (**Supplementary Figure S3**). We found that the optimal dilution fold of U-safe (diluted with ddH_2O) was 20 and the appropriate concentration of PI probes was between 1000 to 500 μg/mL (we used the concentration of 500 μg/mL in this study) although we would think that the fluorescence intensity while using PI fluorescence probes in paper-based test zones could be increased once we obtain the relatively optimal condition of using this probe.

Figure S3. Concentration optimization of fluorescence probes. (a) A solution of U-safe fluorescence nucleic acid probes with a series of dilution was placed into paper-based test zones (on our paper-based diagnostic device); we then recorded the fluorescence intensity profile (of each concentration). We found that the relatively optimal dilution fold to detect the amplified products in paper-based test zones was about 20 (N = 16). (b) A series of diluted solution of PI fluorescence nucleic acid intercalating probes mixed with 100-b.p. DNA ladders was placed into paper-based test zones and then recorded through using an image-recording system, in order to obtain the fluorescence intensity profile (of each concentration). The PI concentration we used in this study was 500 μg/mL (N = 14). We compared the higher concentration group with its next diluted one in (a) and (b). *p < 0.05; **p < 0.01; ***p < 0.001 (Student's t-test), indicated statistically significant differences.



U-safe					
Dilution fold	10	20	30	40	50
Mean± S.D.	57738 ± 5672	53970 ± 5700	35778 ± 3606	22463 ± 1349	18911 ± 1694

PI					
Concentration (μg/mL)	1000	500	250	125	62.5
Mean± S.D.	36100 ± 2671	33823 ± 2583	30578 ± 2893	24731 ± 1560	21455 ± 1138

Table S1. Statistical data of using four types of fluorescence nucleic acid probe to fluorescently detect different DNA ladders (different ladder sizes) with various concentrations in the buffer system (data was presented as Mean ± S.D.).

Ladder size		**1 kilo-base-pair**	
Average concentration	**200 μg/mL**	**100 μg/mL**	**Control**
U-safe	29262 ± 2239	40616 ± 5296	48491 ± 3876
PI	35098 ± 4609	26693 ± 3433	23865 ± 2032
EtBr	17695 ± 1433	22781 ± 2203	31316 ± 3432
DAPI	17711 ± 4525	18363 ± 8432	37491 ± 15072

Ladder size		**100 base-pair**	
Average concentration	**63.5 μg/mL**	**31.75 μg/mL**	**Control**
U-safe	37651 ± 3787	42672 ± 5110	46662 ± 5112
PI	23902 ± 3008	19201 ± 1073	19495 ± 1336
EtBr	26615 ± 2315	31488 ± 4624	36596 ± 3430
DAPI	15493 ± 5416	16183 ± 3322	23655 ± 5145

Ladder size		**25 base-pair**	
Average concentration	**51.4 μg/mL**	**25.7 μg/mL**	**Control**
U-safe	44094 ± 4796	44713 ± 4564	43109 ± 3871
PI	23381 ± 3068	23787 ± 2727	23944 ± 1993
EtBr	33254 ± 4327	33797 ± 3567	32079 ± 3956
DAPI	20355 ± 6132	38559 ± 12181	33673 ± 11259

Table S2. Statistical information of using U-safe fluorescence probe to detect purified amplified products (in human serum and ddH_2O). ($N = 8$; data was presented as Mean ± S.D.)

Purified Amplified Product Concentration	200 μg/mL	20 μg/mL	10 μg/mL
Serum	45731 ± 3981	47793 ± 4828	51833 ± 5749
ddH_2O	43141 ± 3391	52500 ± 3162	58951 ± 3894
Blank	59035 ± 4544	54637 ± 3729	58644 ± 3533

Figure S4. RT-LAMP products detection with PI fluorescence probes. This result showed that PI fluorescence probes can be not used to detect RT-LAMP products (the reverse transcription and amplification of dengue virus serotype-2 RNA with the virus particle concentration of 6000 PFU/mL via RT-LAMP) in paper-based test zones ($N = 8$).

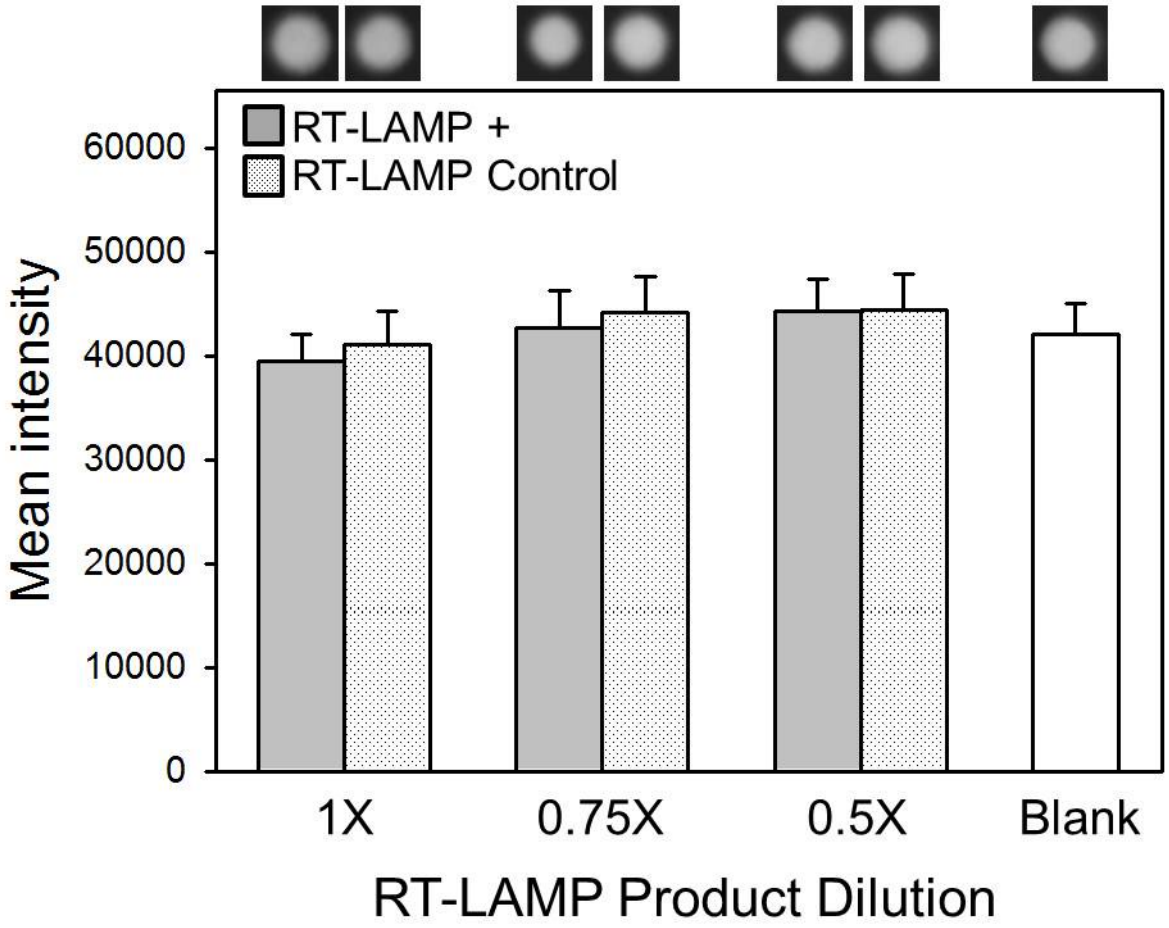


Table S3. Statistical information of using two types of washing reagent to perform the washing step and using U-safe fluorescence probe to detect RT-LAMP products (in ddH_2O without the purification). ($N = 8$; data was presented as Mean ± S.D.)

	ddH_2O wash	**PBS wash**
RT-LAMP +	37470 ± 2512	45514 ± 3899
RT-LAMP Control	44151 ± 3229	49562 ± 4968
Blank	60538 ± 4325	60692 ± 2741

RT-LAMP Product Dilution	**RT-LAMP +**	**RT-LAMP Control**
1X	33130 ± 1999	43198 ± 2539
0.75X	40874 ± 4528	48297 ± 4500
0.5X	49793 ± 4885	54241 ± 4276
Blank	60103 ± 4200	

References

1. M. Parida, K. Horioke, H. Ishida, P. K. Dash, P. Saxena, A. M. Jana, M. A. Islam, S. Inoue, N. Hosaka and K. Morita, *Journal of Clinical Microbiology*, 2005, **43**, 2895-2903.
2. S.-J. Lo, S.-C. Yang, D.-J. Yao, J.-H. Chen and C.-M. Cheng, *IEEE Nanotechnology Magazine*, 2012, **6**, 26-30.