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Supporting Information

A Rapid and Easy-to-Perform Method of Nucleic-Acid Based Dengue Virus Diagnosis Using Fluorescence-Based Molecular Beacons

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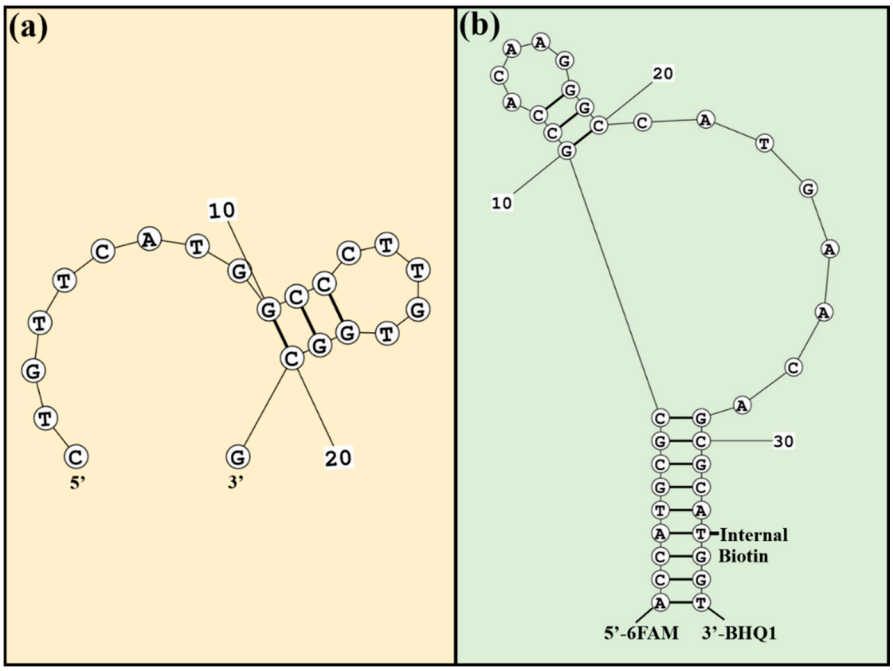

Figure S1. RNAstructure webserver-predicted structure of DENV2-specific target D2C (a) and the DENV2-specific target probe D2P (b) used for detection of D2C. This same D2C target was also used for checking cross-reactivity with the pan-DENV probe.

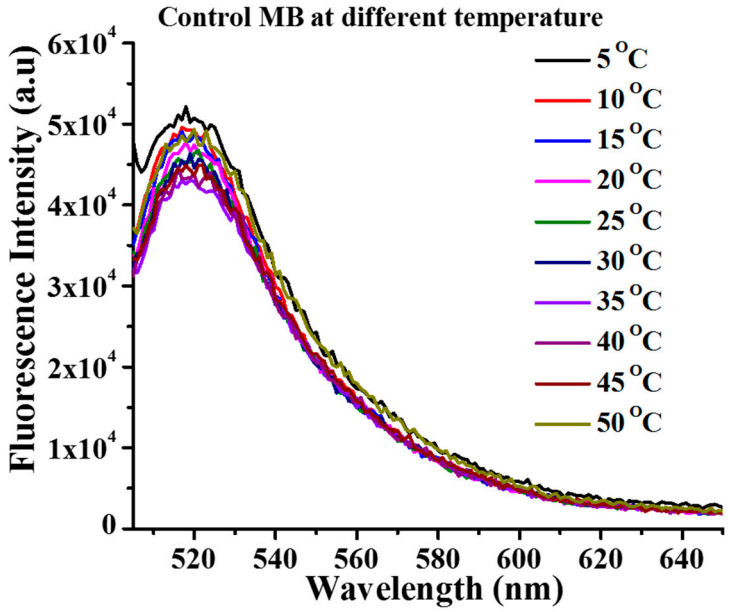

Figure S2. Molecular beacon (MB) standardization, fluorescence intensity of control molecular beacon with increasing temperatures.

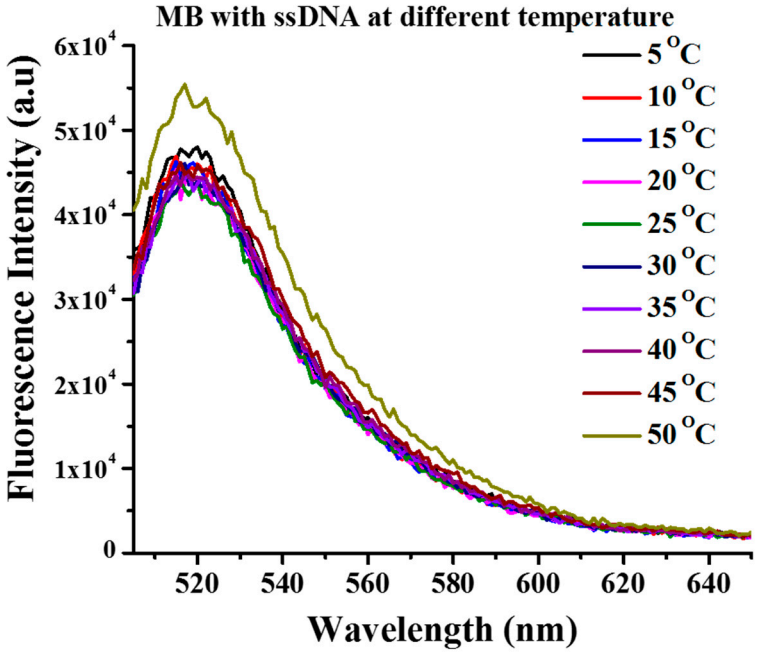

Figure S3. Molecular beacon (MB) standardization, fluorescence intensity of control molecular beacon with target ssDNA with increasing temperatures.

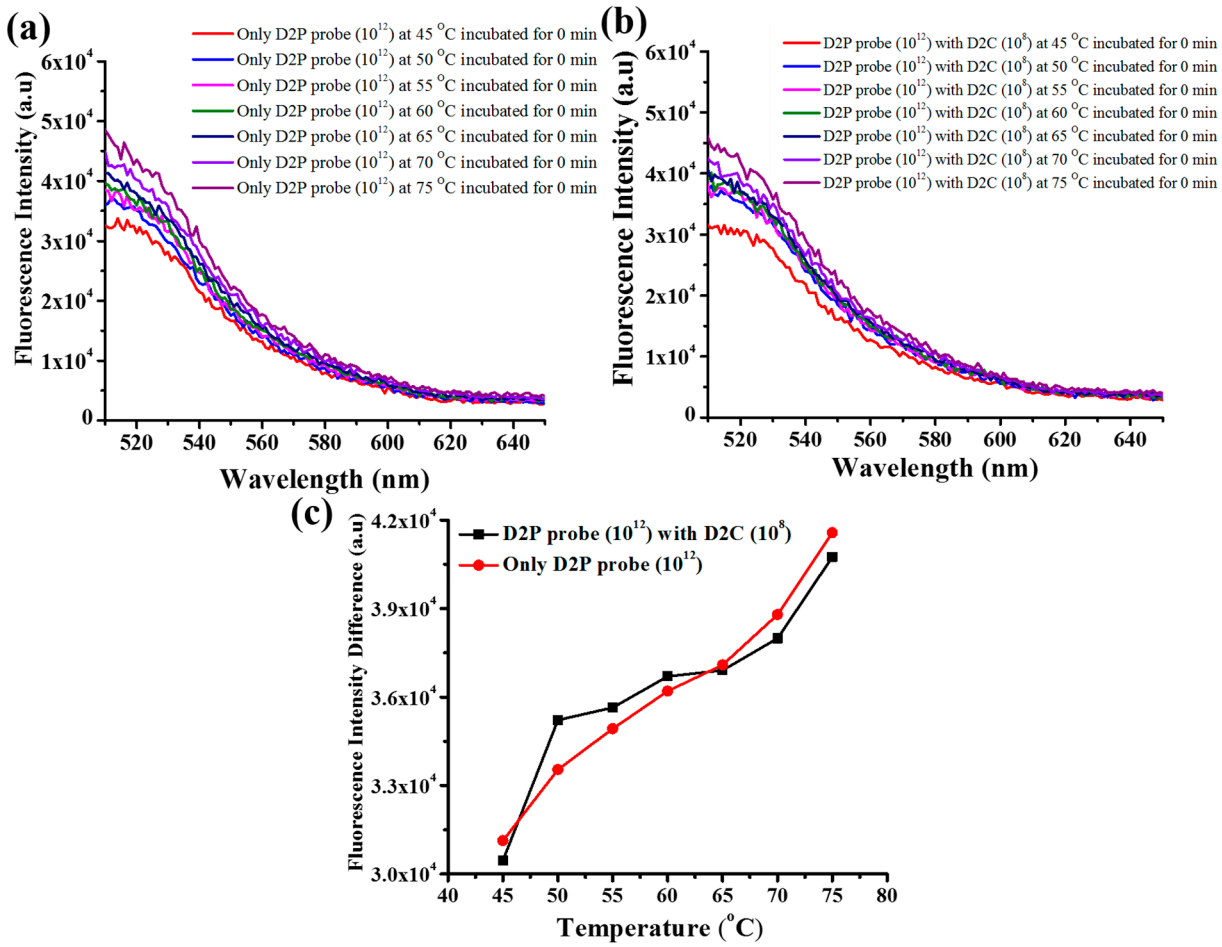

Figure S4. Fluorescence intensity measurement of only D2P probe (a), D2P probe with D2C target sequence (b) with increasing temperature from 45 ^{0}C to 75 ^{0}C. (c) Relative fluorescence increment upon adding D2C target sequence to D2P probe at different temperatures. Maximum fluorescence difference was observed at 50 ^{0}C.

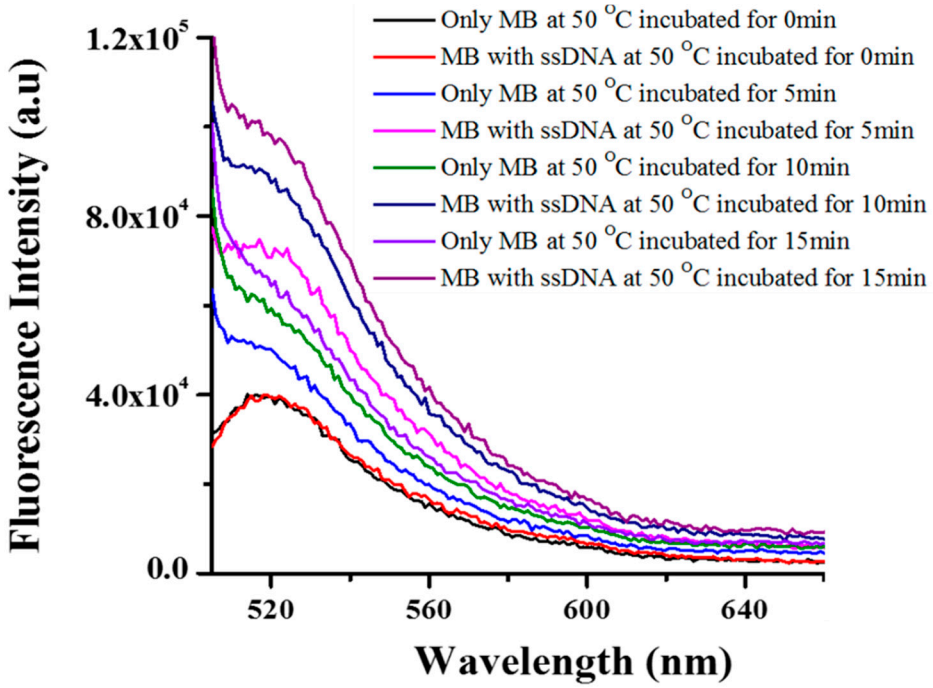

Figure S5. Fluorescence intensity measurement of MB and MB with ssDNA after incubating them for different time periods at 50 ^{0}C.

Fluorometer Settings	Values
Excitation Slit width	1.25 mm
Emission Slit Width	1.25 mm
Voltage	121v
Integration time	0.1sec
Excitation Wavelength	495 nm
Measured Emission range	505-650 nm
Maximum Emission wavelength	519 nm

Figure S6. Fluorometer parameters used to measure the fluorescence intensity of different probes.

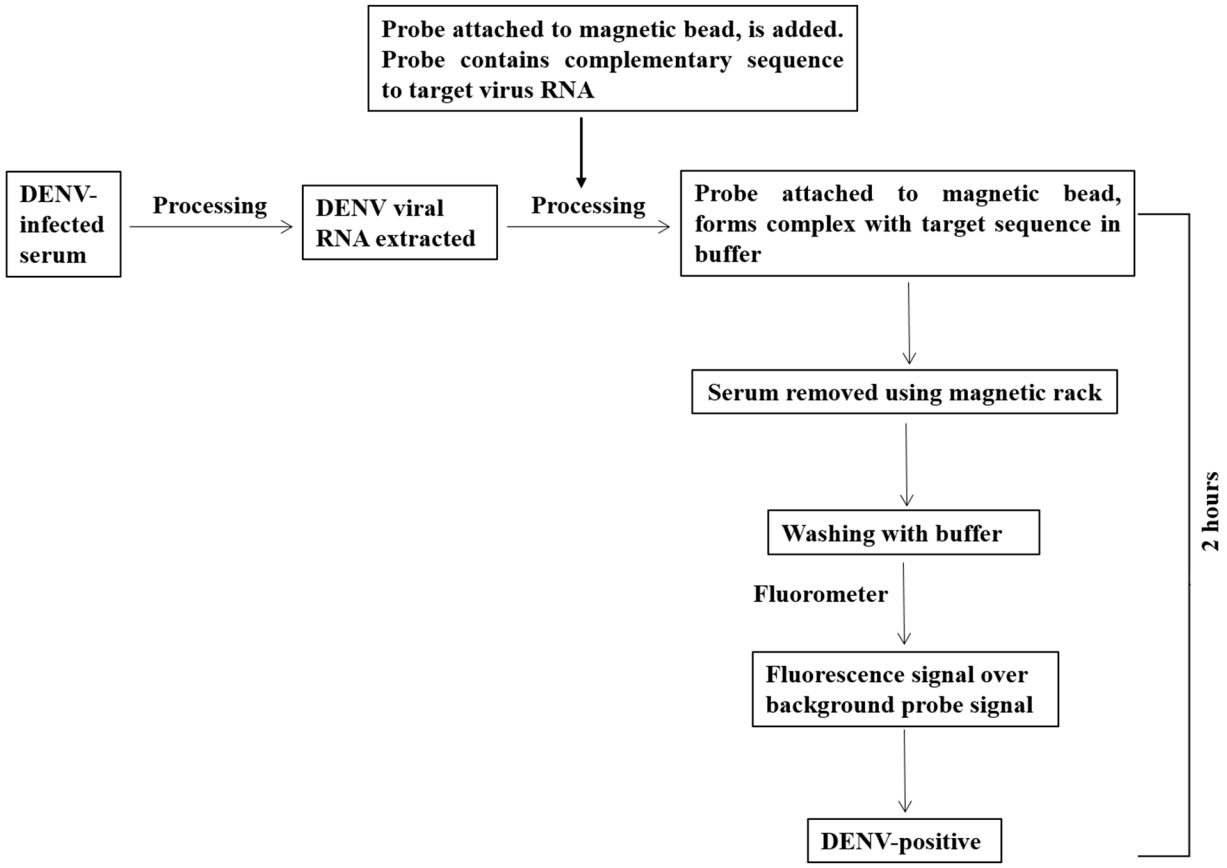

Figure S7. Schematic view of the overall process of the present invention.

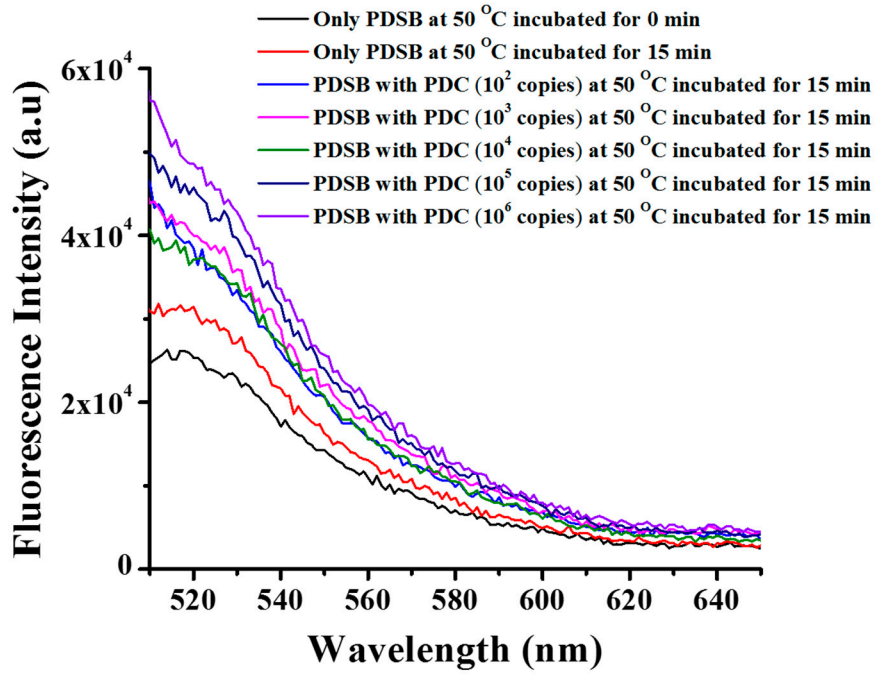

Figure S8. Fluorescence intensity measurement of PDSB probe only and PDSB probe with different concentrations of PDC target at 50^{0}C for 15 min to check the sensitivity of PDSB probe.

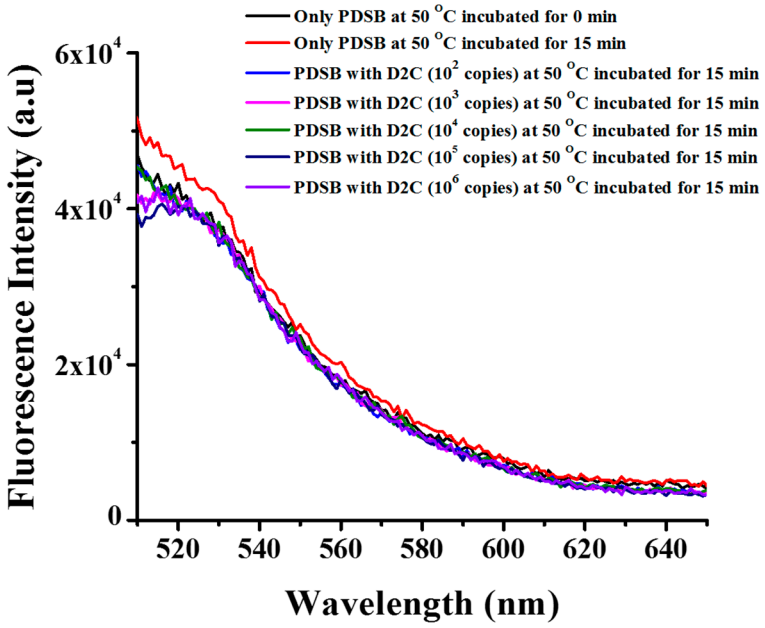

Figure S9. Fluorescence intensity measurement of PDSB probe only and with different concentrations of non-specific target (D2C) to check the specificity of PDSB probe at 50 ^{0}C for 15 min.

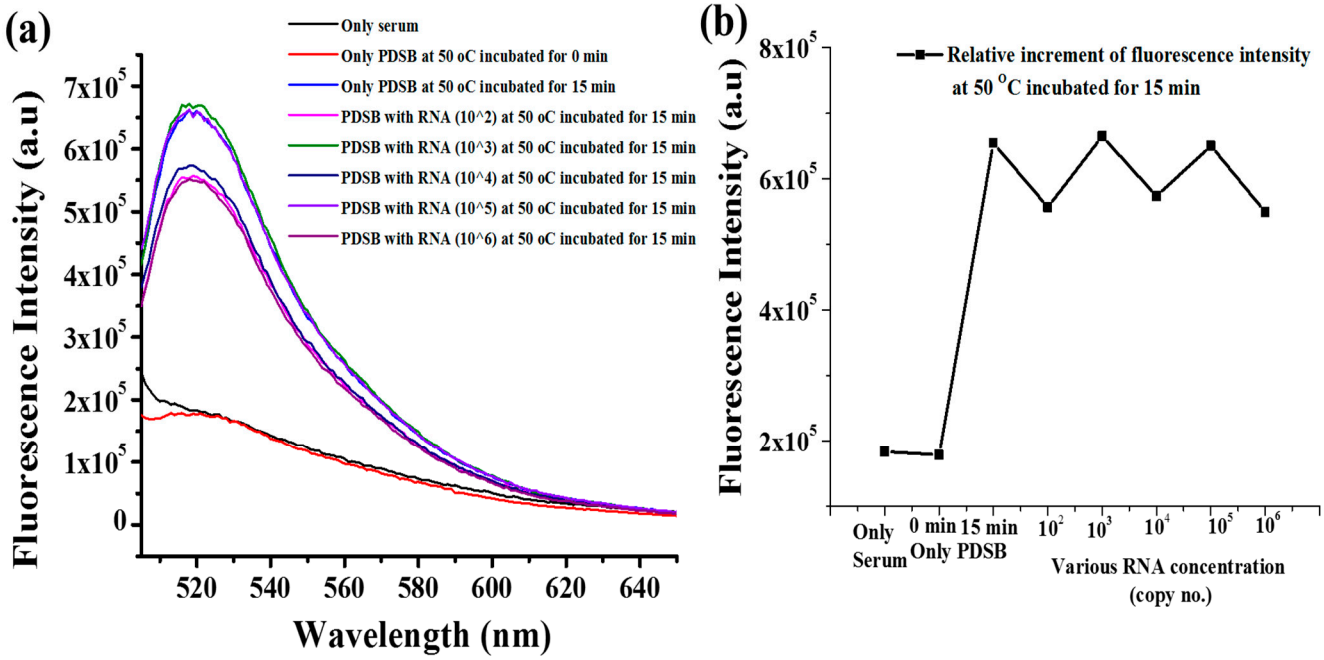

Figure S10. Fluorescence intensity measurement (a) and relative fluorescence increment (b) of PDSB probe (10^{13}) with various concentrations of DENV1 RNA at 50 ^{0}C temperature.

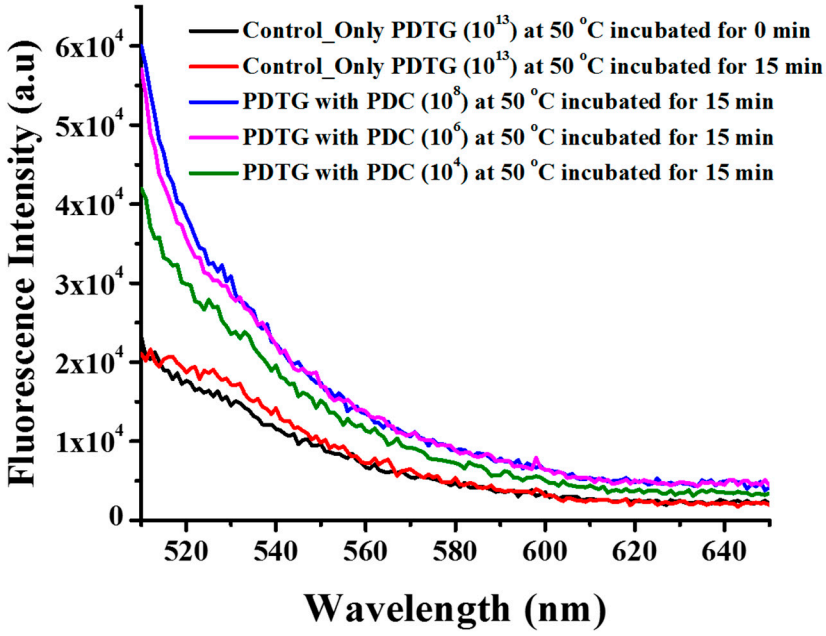

Figure S11. Fluorescence intensity measurement of only biotin tagged pan-dengue probe (PDTG) and PDTG with different copy numbers of target (PDC) at 50 ^{0}C.

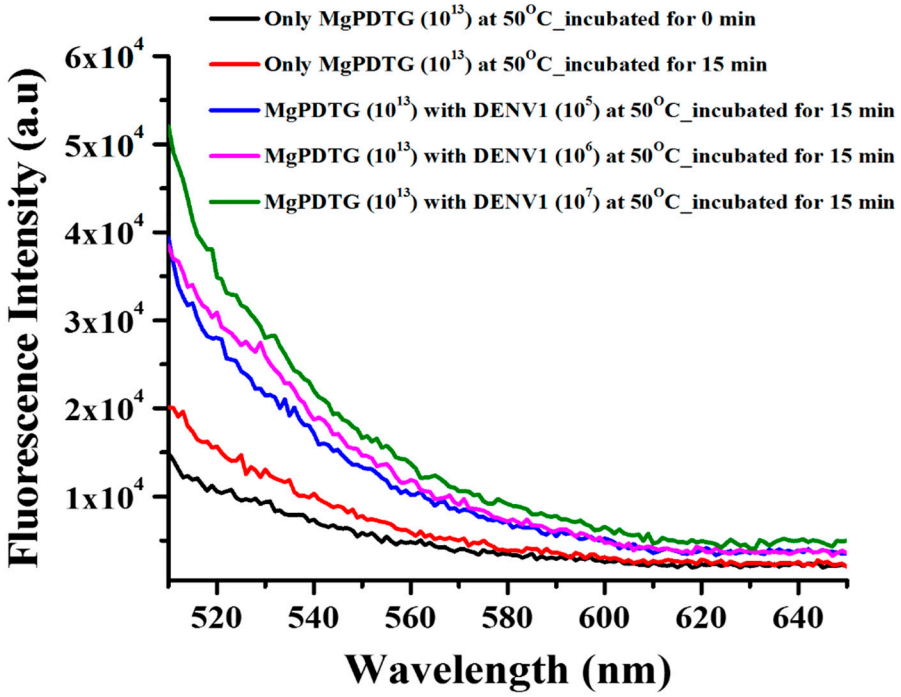

Figure S12. Fluorescence intensity measurement of biotinylated pan-dengue probe immobilized on Strep-MagBeads (Mg-PDTG) only and in presence of DENV1 target sequence at 50 ^{0}C.

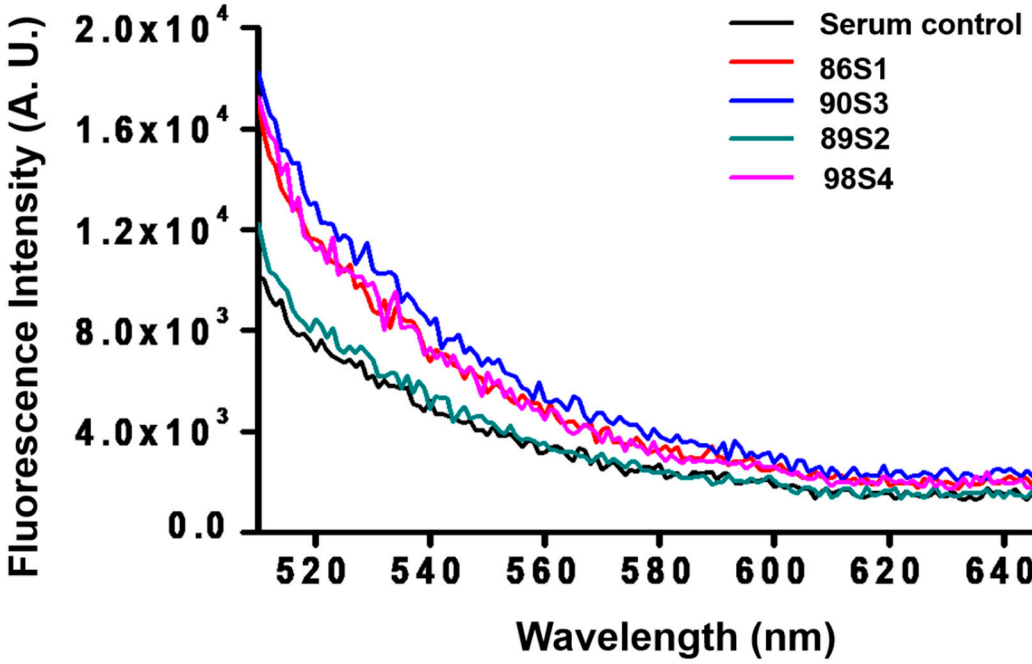

Figure S13. Detection of clinical DENV samples. Fluorescence intensity of various clinical samples measured with Mg-PDTG probe at 50 ^{0}C.

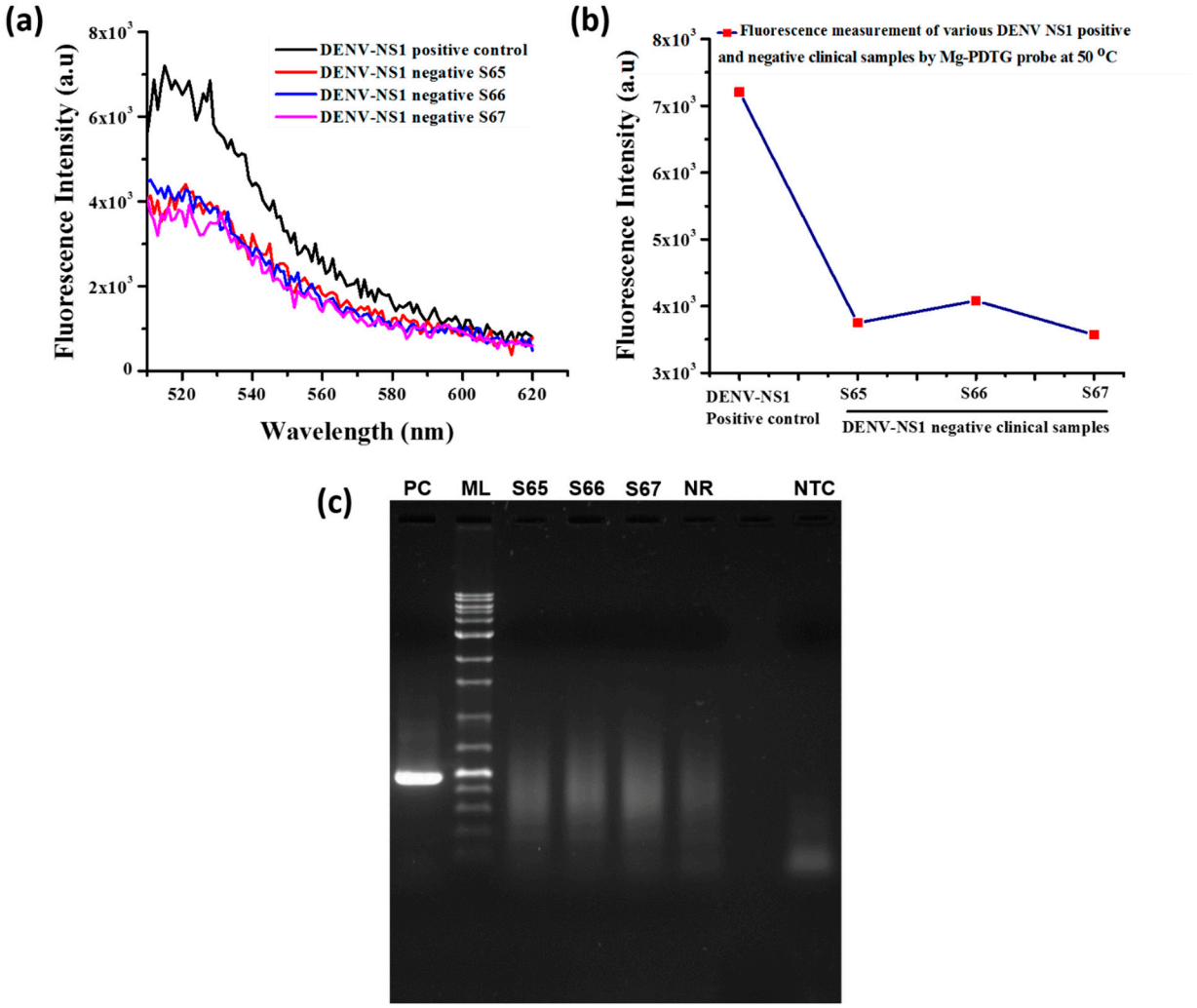

Figure S14. Detection of DENV-negative clinical samples. (a) Fluorescence intensity of a DENV NS1-positive control (PC) with three DENV NS1-negative control clinical samples (S65, S66, and S67) measured with the Mg-PDTG probe at 50 ^{0}C. (b) Quantification graph showing the relative difference in fluorescence intensity between the DENV NS1-positive and DENV NS1-negative clinical samples measured with the Mg-PDTG probe at 50 ^{0}C. (c) Agarose gel (1%) electrophoresis image of the DENV qRT-PCR products, obtained from RNA isolated from the DENV-NS1 positive and negative clinical samples. [PC: DENV NS1-positive control; ML: Molecular weight ladder, same as shown in Figure 3b; NTC: no-template control; NR: not relevant to this study].

	NS1-ELISA method	**qRT-PCR Method**	**Molecular-beacon based detection**
Detection method	Antigen	cDNA (Nucleic acid test)	RNA (Nucleic acid test)
Serotyping	No	Possible	Possible
Sensitivity	Less sensitive than the other two. Sensitivity had been reported to range from 52-86% based on different studies using different commercially available kits [1-7]	Highest sensitivity. Can detect even less than 10 copies of viral genome [8-11]	Can detect up to 10^2 copies but linearity was only perceptible from 10^4 copies and higher
Viral load	Not quantitative	Quantitative	Semi-quantitative
Ease-of-use	Easy to use	Requires trained manpower	Easy to use
Time	2-3 hrs	6-8 hrs	2 hrs

Table S1. Comparison between NS1-ELISA, qRT-PCR and molecular-beacon-based dengue diagnosis.

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