Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTARY MATERIAL

Dengue Serotyping with Label-Free DNA Sensor

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Table S1. Sequences for oligonucleotides used in optimisation for this study.

Oligonucleotide	Sequence (5'-3')
Dengue probe serotype 1	GGG TGG GTG GGG GGA CCC ACC C TCT TGA CTC TCA GAG ACA TAT CAA AGA TTC CAG GGGG TCT TGA CTC TCA GAG ACA TAT CAA AGA TTC CAG GGGG/phos/
Dengue probe serotype 2	GGG TGG GTG GGG GGA CCC ACC C TCT TGA CTC AAG AGA CGT GAG CAG GAA GGA AGG GGG AGC TCT TGA CTC AAG AGA CGT GAG CAG GAA GGA AGG GGG AGC/phos/
Dengue probe serotype 3	GGG TGG GTG GGG GGA CCC ACC C TCT TGA CTC TGA GAG ATA TTT CCA AGA TAC CCG GAG GAG TCT TGA CTC TGA GAG ATA TTT CCA AGA TAC CCG GAG GAG/phos/
Dengue probe serotype 4	GGG TGG GTG GGG GGA CCC ACC C TCT TGA CTC TGG AGG AGA TAG ACA AGA AGG ATG GAG ACC TCT TGA CTC TGG AGG AGA TAG ACA AGA AGG ATG GAG ACC/phos/
Dengue target serotype 1	C CCC CTG GAA TCT TTG ATA TGT CTC TGA
Dengue target serotype 2	GCT CCC CCT TCC TTC CTG CTC ACG TCT CTT
Dengue target serotype 3	CTC CTC CGG GTA TCT TGG AAA TAT CTC TCA
Dengue target serotype 4	GGT CTC CAT CCT TCT TGT CTA TCT CCT CCA
Silver nanocluster strand (positive control)	GGGTGG GTC CCC CCA CCC ACC C

Table S2. Sequences of primers used in reverse transcription of dengue genome RNA.

Oligonucleotide	Sequence (5'-3')
Dengue serotype 1 Forward	TCA GAG ACA TAT CAA AGA TTC CAG GGG G
Dengue serotype 2 Forward	AAG AGA CGT GAG CAA GAA GGA AGG GGG AGC
Dengue serotype 3 Forward	TGA GAG ATA TTT CCA AGA TAC CCG GAG GAG
Dengue serotype 4 Forward	TGG AGG AGA TAG ACA AGA AGG ATG GAG ACC
Dengue Reverse	GT GTC CCA HCC MGC TGT GTC ATC HGC YTA CAT

Table S3. Optimization of assay.

Factor	Unit	Variance
Incubation temperature	°C	50, 53,55,60,65
Incubation time	Hour (hr)	1,2,3,4,5
Concentration of Vent (exo-) DNA polymerase	Unit (U)	0.08,0.10, 0.13,0.17, 0.20
concentration of Nt.BstNBI nicking enzyme	Unit (U)	0.25, 0.40, 055, 0.65, 0.80
concentration of $AgNO_3$	µM	60, 100, 200, 300, 400
concentration of $NaBH_4$	µM	10, 20, 40, 60, 100

Table S4a. Fluorescent intensity of cross-reactivity assays of single probe with all four dengue serotypes.

	Dengue target			
Dengue probe	Serotype 1 (T1)	Serotype 2 (T2)	Serotype 3 (T3)	Serotype 4 (T4)
Serotype 1 (T1)	337.26	165.80	98.64	102.30
Serotype 2 (T2)	106.62	342.47	99.23	104.20
Serotype 3 (T3)	99.62	165.80	377.19	105.98
Serotype 4 (T4)	102.80	157.00	98.33	378.39

Table S4b. Fluorescent intensity of cross-reactivity assays of cocktail probes with all four dengue serotypes.

Cocktail probes assay	Fluorescent intensity (a.u.)
All probes with Dengue target serotype 1 (4P + T1)	259.56
All probes with Dengue target serotype 2 (4P + T2)	263.94
All probes with Dengue target serotype 3 (4P + T3)	261.75
All probes with Dengue target serotype 4 (4P + T4)	267.99
All probes without dengue target (4P only)	75.42

Table S5. Detection of amplified dengue DNA from real samples with both single probe assay and cocktail probes assay.

	Fluorescent intensity (a.u.)	
Target	Single Probe	Cocktail probes
Dengue target serotype 1	692.17	685.15

Dengue target serotype 2	657.32	480.23
Dengue target serotype 3	659.97	494.05
Dengue target serotype 4	669.44	471.80
Negative control	406.58	397.75

Table S6. Melting temperature of dengue DNAs.

Oligonucleotide	Melting temperature Tm (°C)	GC content (%)
Dengue target serotype 1	69	46
Dengue target serotype 2	79	60
Dengue target serotype 3	70	47
Dengue target serotype 4	72	50

*Data was obtained from IDT primer sheet

Table S7. DNA sequences of other flaviviruses used in this study.

Flavivirus	Sequence (5'-3')
Japanese encephalitis virus (JEV)	CTT GCT TTC CTG CTA TGT CAC GGA GGA
Yellow fever virus (YFV)	ACC ATC CAT TGC AGC CAG GTC TCT GA
West Nile virus (WNV)	AGG CCG GGT GCC AAC TTC ACG CA

Table S8. Estimated cost per run for DENV detection using proposed method.

Materials	Estimated Cost per run (USD)
Primer	0.05
DENV detection probe	0.38
Reverse transcriptase kit	9
Vent (exo-) DNA polymerase	0.75
Nt.BstNBI nicking enzyme	0.50
Silver nitrate	0.05
Sodium borohydride	0.025
Total	10.76

*This calculation does not include labour costs and consumables.

Figure S1

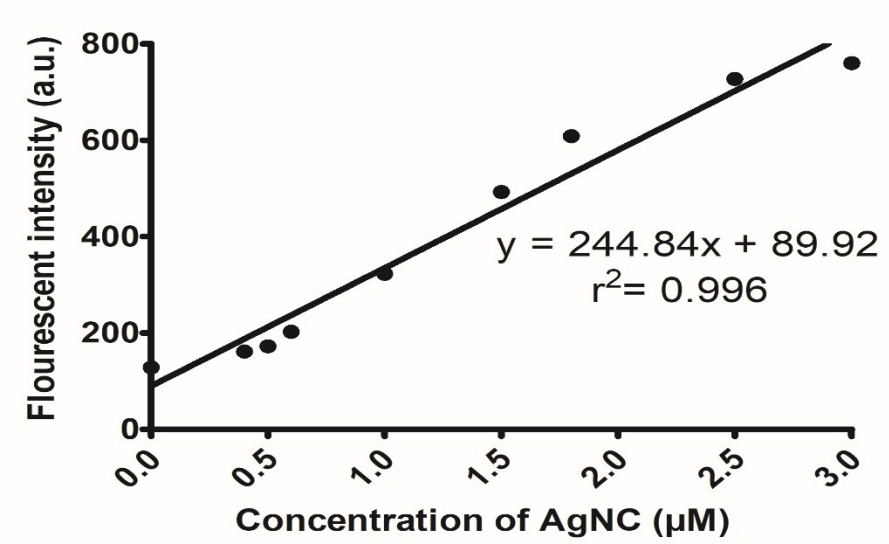

Figure S1. Detection limit of fluorescence spectrophotometer. Detection limit of fluorescence spectrophotometer from 0 μM to 3μM of AgNC strands.

Figure S2

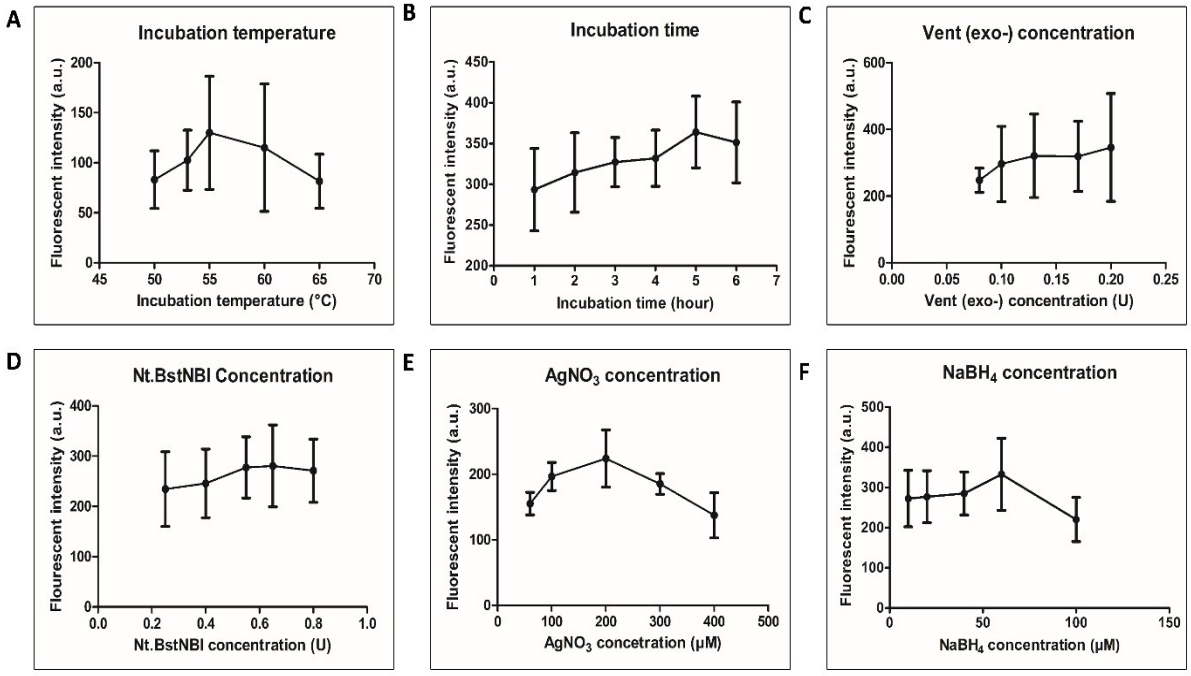

Figure S2. Optimization of various factors for detection. (A) Incubation temperature. (B) Incubation time for amplification and nicking process. (C) Concentration of Vent (exo-) DNA polymerase. (D) Concentration of Nt.BstNBI

nicking enzyme. (E) Concentration of silver nitrate ($AgNO_3$). (F) Concentration of sodium borohydride ($NaBH_4$) reducing agent.

Figure S3

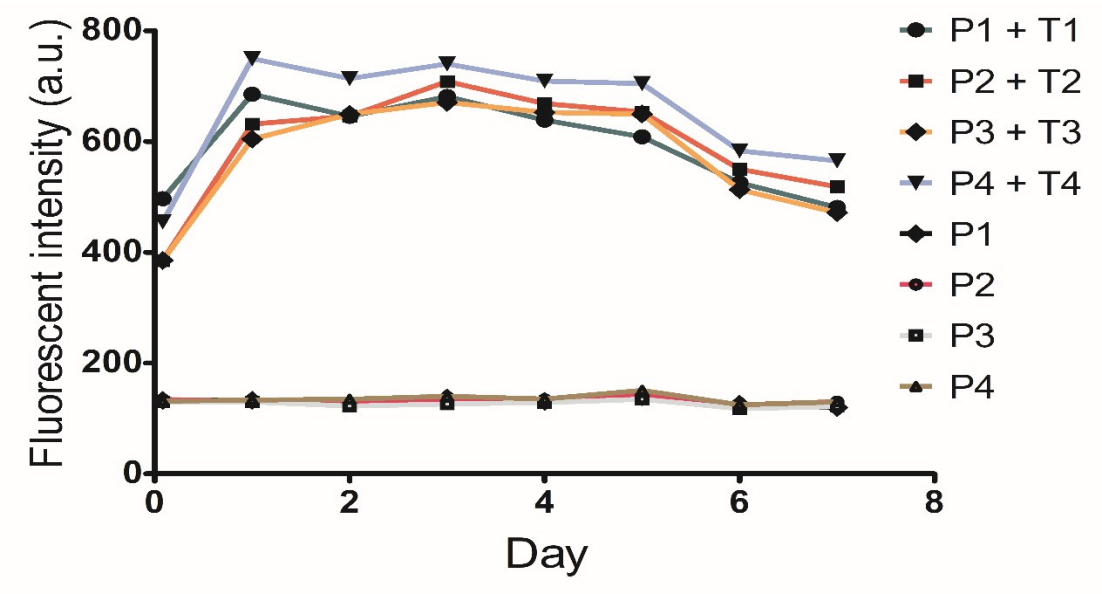

Figure S3. Stability test for silver nanocluster generated. Fluorescent intensity of all dengue serotypes at different time intervals from 2 hours to 7 days.

Figure S4

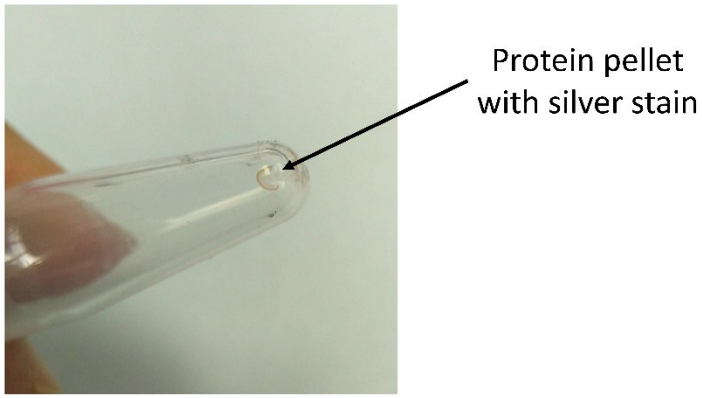

Figure S4. Silver ions from $AgNO_3$ bound to proteins and pelleted down to the bottom of tube after centrifuged.

Figure S5

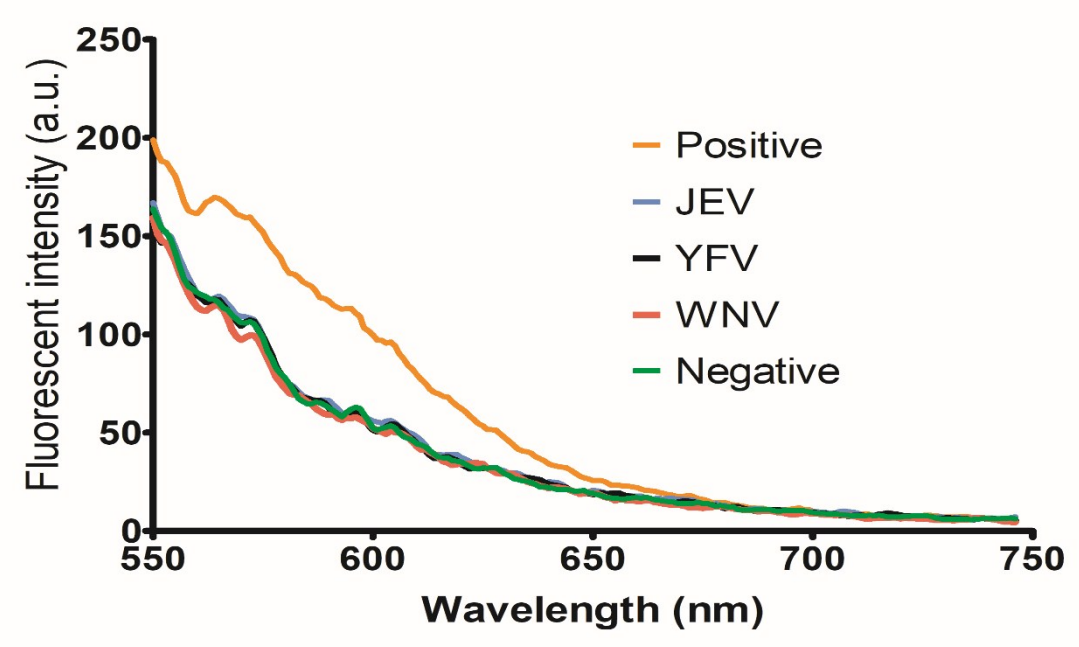

Figure S5. Cross reactivity assay of dengue probes with other flaviviruses. Target DNA was incubated with cocktail dengue probes to perform the assay.