



Figure S1. Diagnostic performances of NS1 MAC-, indirect NS1 IgM, and VLP MAC-ELISAs. DENV2+3 VLP and NS1 antigens were used in the pre-depletion and subsequent NS1 MAC-ELISA. Combined DENV1-4 NS1 antigens were used in the indirect NS1 IgM ELISA. DENV2+3 VLP antigens were also used in the VLP MAC-ELISA. (A) Comparison of the diagnostic performances between NS1 MAC-, indirect NS1 IgM, and VLP MAC-ELISAs as depicted by the fitted ROC curves based on P/N ratio values from 31 primary acute-phase to early convalescent-phase DENV-infected sera and 51 negative control sera. (B) The optimal P/N ratio cutoff (dotted lines) were determined by the magnitude of LR^+ using the formula: $LR^+ = \text{sensitivity}/(100 - \text{specificity})$. All data were obtained from two independent experiments with duplicates. Error bars indicate standard deviations of means.

Table S1. Oligonucleotides used in site-directed mutageneses (SDM) and plasmid constructions.

Primer	Primer sequence (5'-3') ^a	Used for
D1 NS1-His	GGTCTCTGCT <u>CACCACCACCACCACC</u> ACTGATGAGCGG CCGCTC	SDM, insertion of 6xHis tag at D1 NS1 C-terminal
D2 NS1-His	GGTCACAGCCC <u>CACCACCACCACCACC</u> ACTGATGAGCG GCCGCTC	SDM, insertion of 6xHis tag at D2 NS1 C-terminal
D3 NS1-His	AGCCTCAGCA <u>CACCACCACCACCACC</u> ACTGATGAGCG GCCGCTC	SDM, insertion of 6xHis tag at D3 NS1 C-terminal
D4 NS1-His	GGTATCGGCC <u>CACCACCACCACCACC</u> ACTGATGAGCG GCCGCTC	SDM, insertion of 6xHis tag at D4 NS1 C-terminal
SH200-NS1-His F	AGTCATCGCTATTACCATGGTAAATGGCCCGCCTGGCT	SH200 donor plasmid cloning, amplification of NS1-His insert
SH200-D1 NS1-His R	CTGGCAACTAGAAGGCACAGGCGAGGCTGATCAGCG	SH200 donor plasmid cloning, amplification of NS1-His insert
SH200-F	CCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGC	SH200 donor cloning plasmid, amplification of vector backbone
SH200-R	CCATGGTAATAGCGATGACTAATACGTAGATGTACTGC CAAGTAGGAAAG	SH200 donor plasmid cloning, amplification of vector backbone

^a6xHis tag sequences are underlined.

Table S2. Comparison of NS1 MAC-, indirect NS1 IgM, and VLP MAC-ELISAs with RT-PCR in the determination of dengue serostatus.

Test	Result	RT-PCR		AUC (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
		Positive	Negative			
NS1 MAC	Positive	26	2	0.92 (0.85 - 1.00)	83.87 (66.27-94.55)	96.08 (86.54-99.52)
	Negative	5	49			
NS1 IgM	Positive	8	7	0.56 (0.43 - 0.69)*	25.81 (11.86-44.61)	86.27 (73.74-94.30)
	Negative	23	44			
VLP MAC	Positive	30	2	0.99 (0.96 - 1.01)	96.77 (83.30-99.92)	96.08 (86.54-99.52)
	Negative	1	49			

* $p < 0.0001$, significantly different compared to NS1 and VLP MAC.