

Figure S1. Stability of the developed NS1 ELISA kit. Anti-NS1 mAb mixture coated dry stabilized plate, diluted biotinylated pan-anti-NS1 detector mAb in liquid stabilizer and poly-HRP-streptavidin conjugate diluted in liquid conjugate stabilizer were stored at RT (23 ± 2 °C) for 50 days. Two concentrations of DENV 1- 4 recombinant secretory hexameric NS1 antigen i.e., 50 ng/ml and 5 ng/ml were tested on stabilized ELISA kit and OD values were compared with the parallelly tested freshly prepared plate, detector mAb and streptavidin-conjugate.

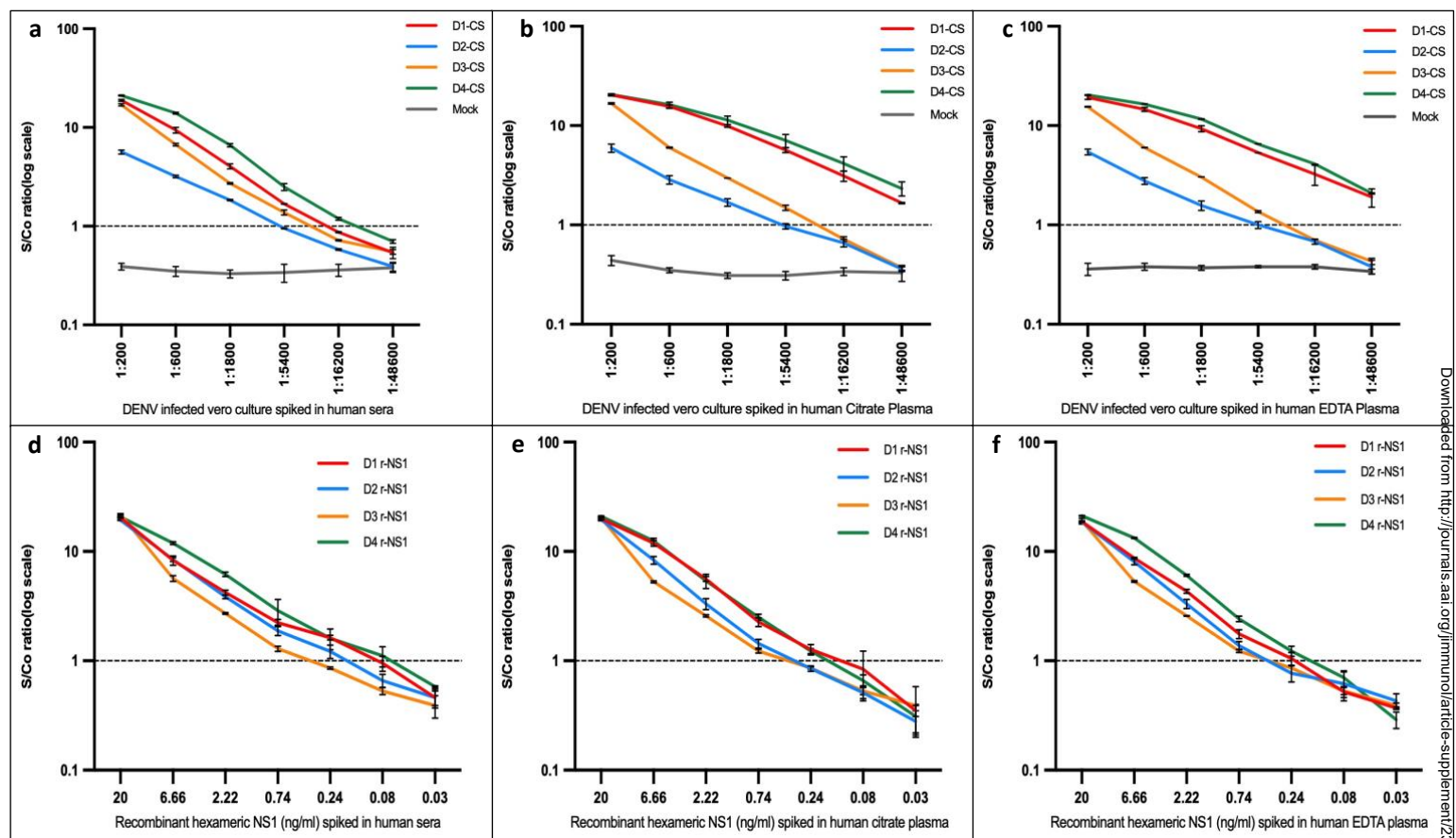


Figure S2. Matrix compatibility of the developed NS1 ELISA. DENV infected Vero cells culture supernatant containing native secreted NS1 (panel a-c) or recombinant hexameric NS1 antigen (panel d-f) from four DENV serotypes diluted in three different matrices i.e., pooled normal human serum (panel a and d), pooled normal human sodium citrate plasma (panel b and e) and pooled normal human EDTA plasma (panel c and f) were used as samples in the developed NS1 ELISA kit. The X-axis represent virus culture supernatants (D1-D4 CS) dilution from 1:200 to 1:48600 or recombinant secretory hexameric NS1 (D1-D4 rNS1) dilution from 20 ng/ml to 0.03 ng/ml in the respective matrices. The dotted line intersecting the y-axis represents the S/Co ratio of 1.

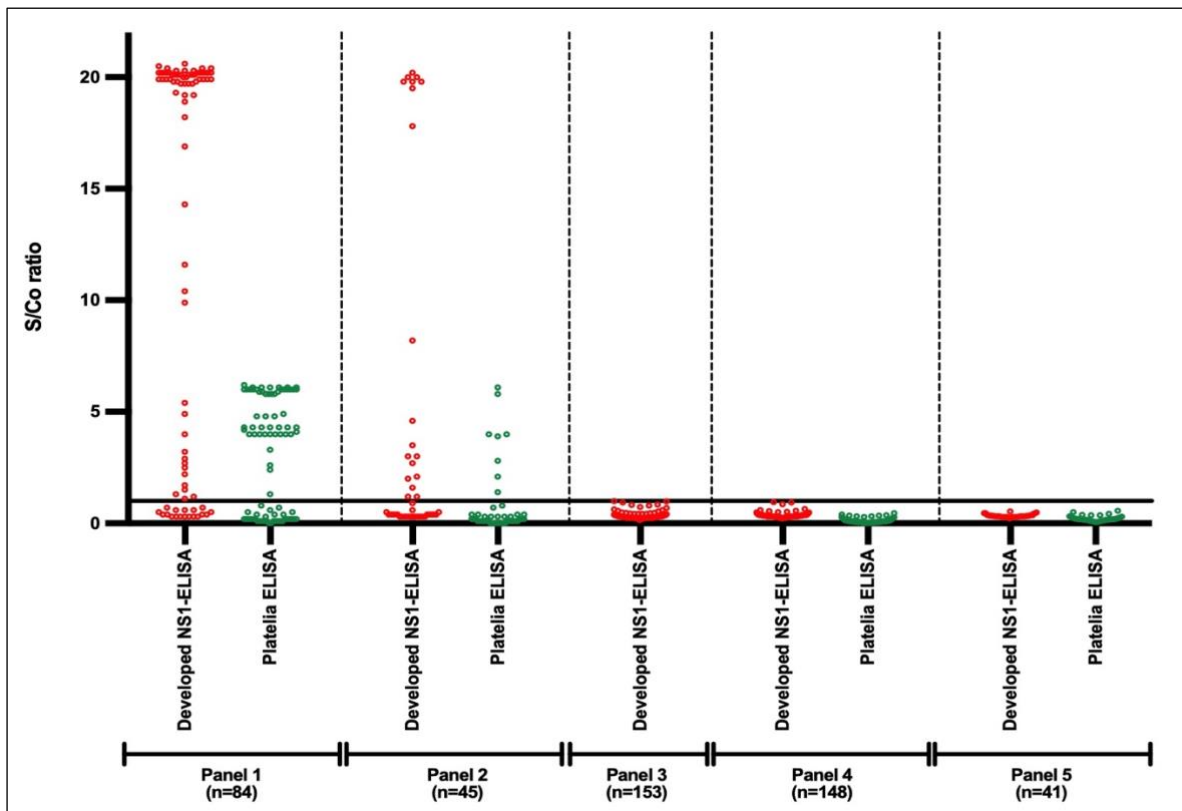


Figure S3. Comparison of the developed NS1 ELISA kit with Platelia NS1 Ag ELISA. The Scatter plots represent the reactivity of serum samples from dengue confirmed, suspected and negative individuals in the developed NS1 ELISA and commercial Platelia NS1 ELISA. The DENV RT-PCR positive samples (Category 1, n=84) and samples negative in DENV RT-PCR but positive for antibodies (IgM/IgG capture ELISA) with clinical suspicion for dengue (category 2, n=45) were run on the tests. The specificities of the tests were determined by running serum samples from individuals positive for scrub typhus but negative for dengue (category 3, n=153), febrile individuals where dengue is ruled out (category 4, n=148) and anti-HCV-Ab or HBsAg positive (category 5, n=41). Panel 3 was not run on Platelia ELISA. The reactivity is shown in terms of the signal-to-cut-off ratio (Y-axis). The line intersecting the Y-axis represents the signal-to-cutoff ratio of 1.0, below which a sample is considered negative in the respective ELISA.

Table S1. Classification of anti-NS1 hybridoma repertoire

S. No.	Category*	Class#	Number of clones	Binding details
1	Pan	1234	1	Secretory DENV-1, 2, 3, and 4 NS1
2	Pan	1234M	2	Secretory DENV-1, 2, 3, and 4 NS1 and , <i>E.coli</i> expressed full-length monomeric NS1
3	Pan	1234WDM	17	Secretory DENV-1, 2, 3, and 4 NS1 and , <i>E.coli</i> expressed wing domain and full-length monomeric NS1
4	Pan	1234ZM	4	Secretory DENV-1, 2, 3, 4 and ZIKV NS1 and , <i>E.coli</i> expressed full-length monomeric NS1
5	Pan	1234ZWD	2	Secretory DENV-1, 2, 3, 4 and ZIKV NS1 and , <i>E.coli</i> expressed wing domain
6	Pan	1234ZWDM	5	Secretory DENV-1, 2, 3, 4 and ZIKV NS1 and , <i>E.coli</i> expressed wing domain and full-length monomeric NS1
7	Subcomplex	12	9	Secretory DENV-1, and 2 NS1
8	Subcomplex	12M	3	Secretory DENV-1, and 2 NS1 and <i>E. coli</i> expressed full-length monomeric NS1
9	Subcomplex	12Z	1	Secretory DENV-1, 2 and ZIKV NS1
10	Subcomplex	13	2	Secretory DENV-1, and 3 NS1
11	Subcomplex	13M	1	Secretory DENV-1, and 3 NS1 and <i>E. coli</i> expressed full-length monomeric NS1
12	Subcomplex	13WD	2	Secretory DENV-1, and 3 NS1 and <i>E. coli</i> expressed wing domain
13	Subcomplex	13WDM	4	Secretory DENV-1, and 3 NS1 and <i>E. coli</i> expressed wing domain and full-length monomeric NS1
14	Subcomplex	14	1	Secretory DENV-1, and 4 NS1
15	Subcomplex	24	1	Secretory DENV-2, and 4 NS1
16	Subcomplex	34	2	Secretory DENV-3, and 4 NS1
17	Subcomplex	34WD	1	Secretory DENV-3, and 4 NS1 and <i>E.coli</i> expressed wing domain
18	Subcomplex	34ZWD	1	Secretory DENV-3, 4, and ZIKV NS1 and <i>E.coli</i> expressed wing domain
19	Subcomplex	123	1	Secretory DENV-1, 2 and 3 NS1
20	Subcomplex	123M	1	Secretory DENV-1, 2 and 3 NS1 and <i>E.coli</i> expressed full-length monomeric NS1
21	Subcomplex	123WDM	1	Secretory DENV-1, 2 and 3 NS1 and <i>E.coli</i> expressed wing domain and full-length monomeric NS1
22	Subcomplex	134	1	Secretory DENV-1, 3, and 4 NS1
23	Subcomplex	134M	2	Secretory DENV-1, 3, and 4 NS1 and <i>E.coli</i> expressed full-length monomeric NS1
24	Subcomplex	134WDM	1	Secretory DENV-1, 3, and 4 NS1 and <i>E.coli</i> expressed wing domain and full-length monomeric NS1
25	Subcomplex	234M	2	Secretory DENV-2, 3 and 4 NS1 and <i>E. coli</i> expressed full-length monomeric NS1
26	Serotype	1	1	Secretory DENV-1 NS1
27	Serotype	1WDM	4	Secretory DENV-1 NS1, and <i>E.coli</i> expressed wing domain and full-length monomeric NS1
28	Serotype	2	1	Secretory DENV-2 NS1
29	Serotype	2WD	3	Secretory DENV-2 NS1, and <i>E.coli</i> expressed wing domain
30	Serotype	2WDM	5	Secretory DENV-2 NS1, <i>E.coli</i> expressed wing domain and full-length monomeric NS1
31	Serotype	3	2	Secretory DENV-3 NS1
32	Serotype	4	7	Secretory DENV-4 NS1
33	Serotype	4M	1	Secretory DENV-4 NS1, and <i>E.coli</i> expressed full-length monomeric NS1
34	Serotype	4WD	1	Secretory DENV-4 NS1, and <i>E.coli</i> expressed wing domain
35	Serotype	4WDM	1	Secretory DENV-4 NS1, and <i>E.coli</i> expressed wing domain and full-length monomeric NS1
36	Serotype	4ZM	1	Secretory DENV-4 and ZIKV NS1, and <i>E.coli</i> expressed full-length monomeric NS1

* Category of mAbs based on recognition of secretory DENV 1-4 NS1.

#Class of mAbs based on differential recognition of secretory NS1 of four DENV serotypes and ZIKV, *E. coli* expressed full-length monomeric DENV 1-4 NS1 and *E. coli* expressed DENV 1-4 NS1 wing domain. "M": *E. coli* expressed monomeric NS1; "WD": *E. coli* expressed wing domain; "Z": secretory NS1 of ZIKV.