

Serum ID	Serum Category	Location of Infection	Days post infection	FRNT50				
				DENV1	DENV2	DENV3	DENV4	ZIKV
DT168	Primary ZIKV	Brazil	> 6 months	25	<20	100	<20	1258
DT172	Primary ZIKV	Colombia	3 months	<20	<20	<20	<20	794
DT206b	Primary ZIKV	Honduras	6 months	<20	<20	<20	<20	23388
DT244b	Primary ZIKV	Puerto Rico	6 months	<20	<20	<20	<20	4860
DT166	Secondary ZIKV	Brazil	> 6 months	630	398	3981	316	1995
ARB15	Secondary ZIKV	Brazil	6 months	1524	666	557	502	14959
ARB24	Secondary ZIKV	Brazil	6 months	126	209	251	106	3918
ARB14	Secondary ZIKV	Brazil	6 months	1524	666	557	502	14959
ARB19	Secondary ZIKV	Brazil	8 months	1379	4218	2270	359	5213
ARB17	Secondary ZIKV	Brazil	5 months	1348	1818	3047	537	2353
DT147	Primary DENV1	Bolivia	2 years	1016	110	62	36	N/A
DT153	Primary DENV1	Guyana	5 years	288	69	58	32	N/A
DT118	Primary DENV3	Nicaragua	1 year	60	32	980	76	N/A
DT125	Primary DENV3	Paraguay	5 years	97	255	1080	49	N/A
ARB27	Primary DENV2	Brazil	8 months	205	1887	238	240	<25
DT033	Primary DENV3	India	>4 months	16	11	426	25	N/A
ARB16	Secondary DENV	Brazil	7 months	797	122	304	72	<25
ARB22	Secondary DENV	Brazil	6 months	89	308	473	82	<25
DT026	Secondary DENV	Dominican Republic	1.5 years	233	236	158	206	N/A
DT146	Secondary DENV	India	1 year	174	439	830	918	N/A
DT141	Secondary DENV	Dominican Republic	8 years	752	845	302	144	N/A
DT160	Secondary DENV	Puerto Rico	1 year	343	1377	462	542	N/A
DT140	Naïve human sera							
DT127	Naïve human sera							

\* Neutralization data is from a different time point, but does not change the serostatus interpretation.

**Table S1. Determination of flavivirus infection status of late convalescent serum specimens (collected >12 weeks post infection).** Serostatus of specimens were categorized into primary or secondary infection using neutralization assay as previously described (1). Serum that had neutralizing antibodies to any one serotype of DENV or to ZIKV with minimal cross-neutralizing antibodies were defined as primary flavivirus infections (meaning that the 50% inhibitory concentration [IC50] for a single DENV serotype or ZIKV was  $\geq 4$ -fold that of any other virus tested). In most cases, the person's travel history corroborated the primary immune status. Serum that had high levels of neutralizing antibody to  $\geq 2$  flaviviruses were defined as secondary (repeat) flavivirus infections. Most secondary infection samples were from persons who had resided in DENV- or ZIKV-endemic countries for  $\geq 5$  years.

Serum ID	Serum Category	Location of Infection	Days post infection	PCR Confirmation	Dengue acute IE	Dengue convalescent IE
5604.12.B.2	Primary ZIKV	Nicaragua	14	ZIKV	20	72
5847.12.B.2	Primary ZIKV	Nicaragua	15	ZIKV	70	45
5749.12.A.2	Primary ZIKV	Nicaragua	20	ZIKV	36	<10
8385.12.B.2	Primary ZIKV	Nicaragua	23	ZIKV	<10	53
7420.12.B.2	Secondary ZIKV	Nicaragua	14	ZIKV	47	31687
4960.4.a	Primary DENV2	Nicaragua	14	DENV2	<10	14
1471.10.a	Primary DENV2	Nicaragua	15	DENV2	<10	<10
1282.8.a	Primary DENV3	Nicaragua	15	DENV3	<10	20
3300.7.a	Primary DENV3	Nicaragua	15	DENV3	<10	88
5412.12.a	Primary DENV2	Nicaragua	15	DENV2	<10	59
9225.13.a	Primary DENV2	Nicaragua	16	DENV2	<10	329
278.4.b	Primary DENV2	Nicaragua	17	DENV2	<10	112
3451.5.a	Primary DENV3	Nicaragua	17	DENV3	19	450
2651.7.a	Primary DENV3	Nicaragua	20	DENV3	<10	813
2983.7.a	Primary DENV3	Nicaragua	23	DENV3	<10	24
2538.4.a	Secondary DENV2	Nicaragua	15	DENV2	29	54738
2551.9.a	Secondary DENV1	Nicaragua	15	DENV1	1370	49924
3.9.A.a	Secondary DENV1	Nicaragua	15	DENV1	71	39515
2453.4.a	Secondary DENV2	Nicaragua	15	DENV2	13	11986
2845.6.a	Secondary DENV3	Nicaragua	15	DENV3	205	>100000
2674.7.a	Secondary DENV3	Nicaragua	16	DENV3	1314	>100000
2416.6.a	Secondary DENV3	Nicaragua	17	DENV3	1381	>100000
2906.4.a	Secondary DENV2	Nicaragua	19	DENV2	34	28815
304.4.A.a	Secondary DENV2	Nicaragua	20	DENV2	182	45923

\*For ZIKV-immune sera, DENV PCR were negative.

**Table S2. Determination of flavivirus infection status of early convalescent serum specimens from Nicaragua (collected between 14 and 23 days post infection).** All the samples were confirmed by PCR testing. IE titers in paired (acute and convalescent) serum specimens were used to determine whether or not a case is primary or secondary infection.

Serum ID	Serum Category	Location of Infection	Days post infection	PCR Confirmation	Serology summary	
					Acute IgM	Acute IgG
GS0437-2	Primary DENV1	Sri	11	DENV1	Neg	Neg
GS0505-2	Primary DENV1	Sri	19	DENV1	Pos	Neg
GS0409-2	Primary DENV1	Sri	24	DENV1	Neg	Neg
GS0517-2	Primary DENV1	Sri	30	DENV1	Neg	Neg
GS0518-2	Primary DENV1	Sri	30	DENV1	Neg	Neg
GS0399-2	Primary DENV1	Sri	60	DENV1	Neg	Neg
GS0523-2	Secondary DENV1	Sri	44	DENV1	Neg	Pos
GS0402-2	Secondary DENV4	Sri	12	DENV4	Pos	Pos
GS0438-2	Secondary DENV4	Sri	18	DENV4	Pos	Pos
GS0504-2	Secondary DENV1	Sri	23	DENV1	Pos	Pos
GS0519-2	Secondary DENV1	Sri	23	DENV1	Pos	Pos
GS0509-2	Secondary DENV1	Sri	34	DENV1	Neg	Pos
GS0521-2	Secondary DENV4	Sri	77	DENV4	Neg	Pos

**Table S3. Determination of flavivirus infection status of early convalescent serum specimens from Sri Lanka (collected between 11 and 77 days post infection).** Sri Lankan samples were confirmed by PCR and serology as primary or secondary DENV infections.

Serum ID	Serum Category	Location of Infection	Days post infection	FRNT50				
				DENV1	DENV2	DENV3	DENV4	ZIKV
LB17	Secondary ZIKV	Colombia	70	23119	16731	23115	6789	11369
LB18	Secondary ZIKV	Colombia	70	27233	19378	18026	614	2483
LB19	Secondary ZIKV	Colombia	70	14886	14702	15625	1490	35233
LB20	Secondary ZIKV	Colombia	70	1155	5424	27366	215	5965
LB22	Secondary ZIKV	Colombia	70	2897	7220	5640	3825	4948
LB23	Secondary ZIKV	Colombia	70	13595	16678	10633	4426	6527

**Table S4. Determination of flavivirus infection status of early convalescent serum specimens from Colombia.** Serostatus of Colombian specimens were categorized into secondary infection because all the specimens had high levels of neutralizing antibody to ZIKV and DENV.

Serum ID	Serum Category	Location of Infection	Days post infection	Reactivity ratio (K394A) / (WT)
LB17	Secondary ZIKV	Colombia	70	1.0
LB18	Secondary ZIKV	Colombia	70	0.8
LB19	Secondary ZIKV	Colombia	70	1.0
LB20	Secondary ZIKV	Colombia	70	1.3
LB22	Secondary ZIKV	Colombia	70	0.9
GS0505-2	Primary DENV1	Sri Lanka	19	1.1
GS0409-2	Primary DENV1	Sri Lanka	24	1.0
GS0399-2	Primary DENV1	Sri Lanka	60	0.8
GS0402-2	Secondary DENV1	Sri Lanka	12	0.9
GS0504-2	Secondary DENV1	Sri Lanka	23	0.8

**Table S5. Characteristics of Z-EDIII<sup>K394</sup> reactivity with early convalescent sera.** The reactivity ratio was calculated from the sandwich ELISA binding of Z-EDIII mutant (K394A) and wild-type Z-EDIII. K394 denotes the Z-EDIII protein in which the lysine residue at position 394 was replaced by alanine.

Serum ID	Serum Category	Location of Infection	PCR Confirmation	Dengue acute IE	Dengue convalescent IE
1115	Primary	Nicaragua	DENV3	<10	27
1117	Primary	Nicaragua	DENV3	<10	62
1122	Primary	Nicaragua	DENV3	<10	22
1128	Primary	Nicaragua	DENV3	<10	<10
1135	Primary	Nicaragua	DENV3	<10	186
1136	Primary	Nicaragua	DENV3	<10	132
1143	Primary	Nicaragua	DENV3	18	662
1149	Primary	Nicaragua	DENV3	<10	1084
1130	Secondary	Nicaragua	DENV3	293	>100000
1133	Secondary	Nicaragua	DENV3	61	7469
1132	Secondary	Nicaragua	DENV3	302	82656
1134	Secondary	Nicaragua	DENV3	3782	45789
1124	Secondary	Nicaragua	DENV3	37	22478
1142	Secondary	Nicaragua	DENV3	265	>100000
1110	Secondary	Nicaragua	DENV3	30	28118
1127	Secondary	Nicaragua	DENV3	9014	15012
1114	Secondary	Nicaragua	DENV3	7459	64390
1095	Secondary	Nicaragua	DENV3	130	>100000
1101	Secondary	Nicaragua	DENV3	1890	16492
1085	Secondary	Nicaragua	DENV3	364	3467
1086	Secondary	Nicaragua	DENV3	3741	>100000
1083	Secondary	Nicaragua	DENV3	150	43260
1187	Secondary	Nicaragua	DENV3	150	13361
1178	Secondary	Nicaragua	DENV3	33	58126

**Table S6. Determination of flavivirus infection status of longitudinal specimens from Nicaraguan patients who had been infected with DENV.** All the samples were confirmed by PCR testing. IE titers in paired (acute and convalescent) serum specimens were used to determine whether or not a case is primary or secondary infection.

## REFERENCE

1. Collins MH, McGowan E, Jadi R, Young E, Lopez CA, Baric RS, Lazear HM, de Silva AM. 2017. Lack of Durable Cross-Neutralizing Antibodies Against Zika Virus from Dengue Virus Infection. *Emerg Infect Dis* 23:773-781.