Supplementary Appendix
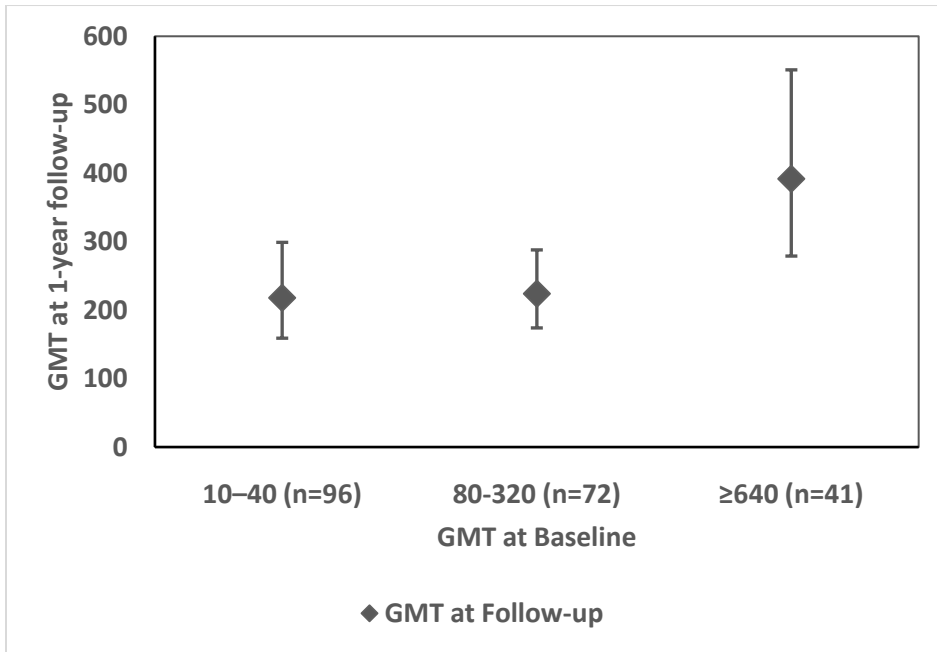
This appendix has been provided by the authors to give readers additional information about their work.

Supplementary Material

Figure S1. Geometric Mean Titer (GMT) at 1 year follow-up in Baseline-Seropositive Participants Stratified by Baseline GMT

For the following two tables, neutralizing antibody titers were determined using $PRNT_{90}$, which is the reciprocal of the endpoint serum dilution that reduces the challenge virus plaque count by 90%. Reading the PRNT at a 90% cut-off decreases the sensitivity of the test (e.g., miss samples containing lower levels of neutralizing antibodies), but improves the specificity by decreasing the likelihood that low level titers caused by cross-reactive antibodies to other related viruses will be detected. Given these characteristics, $PRNT_{90}$ is preferably used for diagnostic purposes. $PRNT_{50}$ are more often used in studies or investigations evaluating pre and post vaccination samples as it provides a more accurate result from the linear portion of the titration curve.[1]

Table S1. Seropositivity Based on $PRNT_{90}$ Results at 1 Month and 1 Year, According to Age Group and Sex*						
	Seropositivity at 1 Month (N = 716)			Seropositivity at 1 Year (N = 684)		
Variable			P Value			P Value
	no./total no.	% (95% CI)		no./total no.	%(95% CI)	
All participants	699/716	98 (96–99)		603/684	88 (86–90)	
Age group			0.08			0.24
2–5 yr	155/162	96 (91–98)		128/151	85 (78–90)	
6–12 yr	188/189	99 (97–100)		160/183	87 (82–91)	
13–49 yr	183/189	97 (93–99)		169/184	92 (87–95)	
≥50 yr	173/176	98 (95–99)		146/166	88 (82–92)	
Sex			0.046			0.34
Male	354/358	99 (97–100)		301/346	87 (83–90)	
Female	345/358	96 (94–98)		302/338	89 (86–92)	

*Seropositivity was defined as a result on $PRNT_{90}$ testing of 10 or higher. P values for the overall comparisons among the subgroups were calculated with the use of Fisher's exact test at 1 month and Chi-squared test at 1 year.

Table S2. Geometric Mean Titer (GMT), Seroconversion or Immune Response at 1 Month, and Seropositivity at 1 Year According to Serostatus at Baseline: Results According to PRNT₉₀ Testing*

Variable	Baseline GMT (95% CI)	Seroconversion or Immune Response at 1 Month (N = 716) no./total no. (%[95% CI])	P Value¶	GMT (95% CI)	P Value‖‖	Seropositivity at 1 Year (N = 684) no./total no. (%[95% CI])	P Value‡	GMT (95% CI)	P Value‖‖
Seronegative at baseline									
All participants	NA	531 / 548 (97 [95–98])	NA	285 (242–337)	NA	452 / 527 (86 [83–88])	NA	29 (25–34)	NA
Age group (yr)			0.02		<0.001		0.005		<0.001
2–5	NA	82 / 89 (92 [85–96])		110 (73–166)		62 / 83 (75 [64–83])		13 (9–18)	
6–12	NA	142 / 143 (99 [96–100])^		247 (189–324)		117 / 139 (84 [77–89])		23 (17–31)	
13–49	NA	165 / 171 (96 [93–98])		488 (355–671)‖		152 / 167 (91 [86–94])^^		44 (35–55)‖	
≥50	NA	142 / 145 (98 [94–99])		314 (230–428)‡		121 / 138 (88 [81–92])		38 (29–51)‖	
Sex			0.02		0.93		0.24		0.19
Male		280 / 284 (99 [96–99])		295 (238–366)		232 / 276 (84 [79–88])		27 (22–33)	
Female	------	251 / 264 (95 [92–97])		275 (214–355)		220 / 251 (88 [83–91])		33 (27–40)	
Seropositive at baseline									
All participants	48 (39–59)	95 / 168 (57 [49–64])	NA	302 (242–378)	NA	151 / 157 (96 [92–98])	NA	57 (46–70)	NA
Age group (yr)			<0.001		0.27		0.29		0.82
2–5	37 (29–48)	47 / 73 (64 [53–74])		314 (221–446)		66 / 68 (97 [90–99])		60 (44–81)	
6–12	38 (27–53)	35 / 46 (76 [62–86])		345 (236–504)		43 / 44 (98 [88–100])		61 (42–88)	
13–49	122 (61–244)	6 / 18 (33 [16–56])		346 (189–631)		17 / 17 (100 [82–100])		53 (29–97)	
≥50	70 (37–133)	7 / 31 23 (11–40)¥		209 (109–402)		25 / 28 (89 [73–96])		46 (22–98)	
Sex			0.37		0.27		0.23		0.90

Male	39 (29–52)	45 / 74 (61 [49–71])	342 (248–472)	69 / 70 (99 [92–100])	59 (44–78)
Female	56 (42–74)	50 / 94 (53 [43–63])	274 (200–375)	82 / 87 (94 [87–98])	55 (40–76)

* Seropositivity was defined as a result on $PRNT_{90}$ testing of 10 or higher. Note: Fifty-five participants were seronegative at baseline using a $PRNT_{90}$ but were seropositive at baseline using a $PRNT_{50}$. NA denotes not applicable

¶ P values for the differences in seroconversion at 1-month follow-up were calculated using Fisher's exact test. P values for the differences in immune response at 1 month were calculated using the Cochran-Manten-Haenszel test, adjusting for baseline titer.

⫫ P values for the global test of differences in the GMT at follow-up were calculated with the Kruskal Wallis test (age group) and the Wilcoxon rank-sum test (sex)

‡ P values for the differences in seropositivity at 1 year were calculated using the Chi-square test (for baseline seronegative participants) and Fisher's exact test (for baseline seropositive participants)

^ Significantly more likely to demonstrate an immune response compared to 2–5 year age group (Fisher's exact test using the Bonferroni correction with adjusted alpha = 0.0083)

^^ Significantly more likely to demonstrate an immune response compared to 2–5 year age group (Chi Square test using the Bonferroni correction with adjusted alpha = 0.0083)

¥ Significantly less likely to demonstrate an immune response compared to 2–5 year and 6–12 year age groups (Cochran-Mantel-Haensel test adjusted for baseline titer and using the Bonferroni correction with adjusted alpha = 0.0083)

‡ Significantly higher titers than 2-5 year age group (Wilcoxon rank-sum test using the Bonferroni correction with adjusted alpha = 0.0083)

‖ Significantly higher titers than 2-5 year and 6-12 year age group (Wilcoxon rank-sum test using the Bonferroni correction with adjusted alpha = 0.0083)

Reference

1. WHO 2007 Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses. Available at: https://apps.who.int/iris/bitstream/handle/10665/69687/who_ivb_07.07_eng.pdf;jsessionid=19BF765D9B3FDAD85EF3F217B0165953?sequence=1. Accessed on 6 February 2019.