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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: DiazGranados CA, Bonaparte M, Wang H, et al. Accuracy and efficacy of pre-dengue vaccination screening for previous dengue infection with five commercially available immunoassays: a retrospective analysis of phase 3 efficacy trials. *Lancet Infect Dis* 2020; published online Nov 16. https://doi.org/10.1016/S1473-3099(20)30695-2.

Supplementary Materials

Table S1. Details of the immunoassays tested

Name	EUROIMMUN anti-dengue virus IgG ELISA	Panbio® Dengue IgG Indirect ELISA[1]	TELL ME FAST Dengue IgG/IgM Combo Test Device[2]	SD BIOLINE Dengue IgG/IgM WB[1]	*OnSite* Dengue IgG/IgM Combo Rapid Test CE[1]
Assay Type	**ELISA**	**ELISA**	**RDT**	**RDT**	**RDT**
Company	Euroimmun	Alere (Abbott)	Biocan Diagnostics, Inc	Alere (Abbott)	CTK Biotech, Inc
Test catalogue number	EI 266b-9601 G	10PE30	B803C	11FK20	R0061C
Package insert,	EI_266bG_A_UK_C04,	01PE30 Rev.1,	Rev 02/2016/RUO	11FK20-02-1,	PI-R0061C Rev. I1.0,
Date on insert	08-09-2011	08-2013	02-2016	01-2009	21-01-2018
Assay principle	ELISA	ELISA	Lateral flow immunochromatography	Lateral flow immunochromatography	Lateral flow immunochromatography
Dengue antigen	Highly purified DENV2 virus antigens	Purified dengue virus antigen	Recombinant dengue antigen	Dengue recombinant envelope antigen	Dengue recombinant envelope antigens
Class of Ig detected	IgG	IgG	IgG, IgM	IgG, IgM	IgG, IgM
Specimen type	Serum or plasma	Serum	Whole blood serum or plasma	Whole blood, serum or plasma	Whole blood, serum or plasma
Sample volume required	10 µL	10 µL	10 µL	10 µL	5 µL
Time to result from blood sample	minimum 2·5 hrs*	minimum 2·5 hrs*	15–20 minutes†	15–20 minutes†	20–25 minutes†
Number of readers	NA	NA	1	2‡	1
Storage temp	2–8 ºC	2–8 ºC	2–30 ºC	Room temperature	2–30 ºC
Cross-reactivity, % (number positive/number tested)¶[1,2]					
Zika	8 (2/26)	34 (14/41)	3 (1/38)	7 (3/41)	0 (0/41)
Japanese Encephalitis	0 (0/40)	3 (1/37)	0 (0/40)	0 (0/37)	0 (0/37)
Yellow Fever	0 (0/49)	0 (0/57)	4 (2/49)	0 (0/57)	0 (0/57)

West Nile virus	10 (6/59)	51 (30/59)	0 (0/59)	7 (4/59)	0 (0/59)

*Based on inclusion of incubation times from the assay protocol and excluding time for sample transportation to the laboratory, batch analysis and reporting to the clinician (two visits for potential vaccine recipients). †Serum/plasma needs to be separated by centrifugation before testing adds approximately 30 minutes to test. ‡Two readers were used, with pre-defined plan to score discordant readings (+/-) as a positive test, sensitivity analyses were also performed scoring discordant readings as negative. ¶ Percentage of dengue seronegative samples that were incorrectly classified by the immunoassay.

ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; RDT, rapid diagnostic test; temp, temperature

[1]Data for cross-reactivity sample selection and results for the *OnSite*-RDT, SD BIOLINE-RDT and Panbio-ELISA reported in Bonaparte M, Zheng L, Garg S, et al. Evaluation of rapid diagnostic tests and conventional enzyme-linked immunosorbent assays to determine prior dengue infection. *Journal of Travel Medicine* 2019; 26; [2]data for the TELL ME FAST-RDT reported in Bonaparte M, Huleatt J, Hodge S, et al. Evaluation of dengue serological tests available in Puerto Rico for identification of prior dengue infection for pre-vaccination screening. *Diagnostic microbiology and infectious disease* 2019:114918. Cross-reactivity data for the EUROIMMUN-ELISA has not previously been published.

Current availability of the ELISAs and RDTs: EUROIMMUN-ELISA is registered in Latin America (Brazil, Colombia, Cuba, Mexico, Peru, and Venezuela), Asia (Australia, Thailand and India) and elsewhere (Belarus, Canada, European Union [EU], Macedonia, Russia, Saudi Arabia, Ukraine and Serbia); the Panbio-ELISA is registered in Latin America (Argentina, Brazil, Colombia, Ecuador, Guatemala, Mexico, Paraguay, and Peru), Asia (Australia, Cambodia, Indonesia, Malaysia, Singapore, and Thailand), and elsewhere (EU, Germany, Israel, Italy, and Portugal); the TELL ME FAST-RDT is registered in Latin America (Costa Rica, Ecuador, El Salvador, Guatemala, Guyana, Paraguay, and Trinidad & Tobago), Asia (Laos, Nepal, Philippines, and Pakistan), and elsewhere (Angola, EU, Iraq, Kenya, Lebanon, Tanzania, and Tunisia); the SD BIOLINE-RDT is registered in Latin America (Brazil and Colombia), Asia (Australia, Bangladesh, Cambodia, India, Indonesia, Malaysia, Singapore, and Thailand), and elsewhere (Ethiopia, EU, Germany, Italy, Russia, Saudi Arabia and Tanzania); the *OnSite*-RDT is registered in Latin America (Argentina, Bolivia, Brazil, Colombia, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Paraguay, Peru, and Venezuela), Asia (Bangladesh, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam), and elsewhere (EU, Mozambique, and Saudi Arabia).

Table S2. Dengue serostatus by the reference algorithm

Group	Serostatus based on reference tests			Interpretation
	$PRNT_{90}$*	**$PRNT_{50}$***	**NS1 IgG ELISA†**	
1	Negative	Negative	Negative	Reference seronegative
2	Negative	Positive	Negative	Reference seronegative
3	Negative	Negative	Low Positive	Reference seronegative
4	Negative	Positive	Low Positive	Reference seropositive
5	Negative	Any‡	High Positive	Reference seropositive
6	Positive	Positive	Any‡	Reference seropositive

*$PRNT_{50}$ & $PRNT_{90}$ positive is defined by titer ≥10 (1/dil) against ≥1 dengue serotype; †Dengue NS1 IgG ELISA results are classified as negative (titer <9 EU/ml), low positive (≥9 to <50 EU/ml) and high positive (≥50 EU/ml). ‡Positive or negative.

ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; NS1, non-structural protein 1; PRNT, plaque reduction neutralization test.

Table S3. Baseline dengue serostatus in the immunogenicity subsets of CYD14 and CYD15 by the reference algorithm and treatment assignment

Reference Group	Participants by group, n (%)		
	Total population (N=3962)	**CYD-TDV Group (N=2643)**	**Placebo Group (N=1319)**
1	875 (22·1)	568 (21·5)	307 (23·3)
2	208 (5·2)	161 (6·1)	47 (3·6)
3	144 (3·6)	96 (3·6)	48 (3·6)
Seronegative	**1227 (31·0)**	**825 (31·2)**	**402 (30·5)**
4	59 (1·5)	45 (1·7)	14 (1·1)
5	29 (0·7)	19 (0·7)	10 (0·8)
6	2647* (66·8)	1754 (66·4)	893 (67·8)
Seropositive	**2735 (69·0)**	**1818 (68·8)**	**917 (69·5)**

Of the above samples with dengue reference testing, ≤3962 were tested by one or more immunoassays.
*5 participants in Group 6 did not have evaluable results in any of the immunoassays and so are not included in this total.

Table S4. Baseline characteristics of the EUROIMMUN-ELISA test positive participants

	CYD-TDV group (N=1575)	Placebo group (N=796)
Sex, male, n (%)	746 (47·4)	411 (51·6)
Mean age, years (SD)	11·4 (3·1)	11·5 (3·0)
Age group, n (%)		
≥9 years	1336 (84·8)	679 (85·3)
<9 years	239 (15·2)	117 (14·7)
≥6 years	1446 (91·8)	738 (92·7)
Ethnic origin, n (%)		
Asian	612 (38·.9)	324 (40·7)
Black	34 (2·2)	11 (1·4)
Caucasian	37 (2·3)	22 (2·8)
Hispanic	0 (0)	0 (0)
American Indian/Alaska native	100 (6·3)	46 (5·8)
Native Hawaiian/Pacific Islander	0 (0)	0 (0)
Other	792 (50·3)	393 (49·4)

Data pooled from CYD14 and CYD15.

Table S5. Baseline characteristics of the Panbio-ELISA test positive participants

	CYD-TDV group (N=1637)	Placebo group (N=833)
Sex, male, n (%)	786 (48·0)	424 (50·9)
Mean age, years (SD)	11·4 (3·1)	11·4 (3·1)
Age group, n (%)		
≥9 years	1387 (84·7)	698 (83·8)
<9 years	250 (15·3)	135 (16·2)
≥6 years	1503 (91·8)	768 (92·2)
Ethnic origin, n (%)		
Asian	643 (39·3)	346 (41·5)
Black	35 (2·1)	13 (1·6)
Caucasian	41 (2·5)	27 (3·2)
Hispanic	0 (0)	0 (0)
American Indian/Alaska native	101 (6·2)	46 (5·5)
Native Hawaiian/Pacific Islander	0 (0)	0 (0)
Other	817 (49·9)	401 (48·1)

Data pooled from CYD14 and CYD15.

Table S6. Baseline characteristics of the TELL ME FAST-RDT test positive participants

	CYD-TDV group (N=935)	Placebo group (N=493)
Sex, male, n (%)	437 (46·7)	254 (51·5)
Mean age, years (SD)	11·7 (2·9)	11·7 (2·9)
Age group, n (%)		
≥9 years	827 (88·4)	437 (88·6)
<9 years	108 (11·6)	56 (11·4)
≥6 years	874 (93·5)	461 (93·5)
Ethnic origin, n (%)		
Asian	300 (32·1)	173 (35·.1)
Black	17 (1·8)	6 (1·2)
Caucasian	26 (2·8)	15 (3·0)
Hispanic	0 (0)	0 (0)
American Indian/Alaska native	81 (8·7)	34 (6·9)
Native Hawaiian/Pacific Islander	0 (0)	0 (0)
Other	511 (54·7)	265 (53·8)

Data pooled from CYD14 and CYD15.

Table S7. Baseline characteristics of the SD BIOLINE-RDT test positive participants

	CYD-TDV group (N=1289)	Placebo group (N=649)
Sex, male, n (%)	623 (48·3)	330 (50·8)
Mean age, years (SD)	11.5 (3·)	11.6 (3·1)
Age group, n (%)		
≥9 years	1104 (85·6)	560 (86·3)
<9 years	185 (14·4)	89 (13·7)
≥6 years	1186 (92·0)	604 (93·1)
Ethnic origin, n (%)		
Asian	447 (34·7)	235 (36·2)
Black	36 (2·8)	12 (1·8)
Caucasian	44 (3·4)	28 (4·3)
Hispanic	0 (0)	0 (0)
American Indian/Alaska native	89 (6·9)	39 (6·0)
Native Hawaiian/Pacific Islander	0 (0)	0 (0)
Other	673 (52·2)	335 (51·6)

Data pooled from CYD14 and CYD15.

Table S8. Baseline characteristics of the *OnSite*-RDT test positive participants

	CYD-TDV group (N=867)	Placebo group (N=442)
Sex, male, n (%)	400 (46·1)	221 (50·0)
Mean age, years (SD)	11·6 (3·0)	11·7 (3·0)
Age group, n (%)		
≥9 years	759 (87·5)	388 (87·8)
<9 years	108 (12·5)	54 (12·2)
≥6 years	804 (92·7)	413 (93·4)
Ethnic origin, n (%)		
Asian	312 (36·0)	165 (37·3)
Black	20 (2·3)	5 (1·1)
Caucasian	21 (2·4)	10 (2·3)
Hispanic	0 (0)	0 (0)
American Indian/Alaska native	44 (5·1)	26 (5·9)
Native Hawaiian/Pacific Islander	0 (0)	0 (0)
Other	470 (54·2)	236 (53·4)

Data pooled from CYD14 and CYD15.

Table S9. Vaccine efficacy against symptomatic VCD in test-positive subjects by different age cut offs (Month 0 – Month 25)

	CYD-TDV group		Placebo group		Vaccine Efficacy (95% CI)
	n/N	Density incidence (95% CI)	n/N	Density incidence (95% CI)	
Participants ≥9 years					
EUROIMMUN-ELISA	5/1336	0·2 (0·1, 0·4)	33/679	2·5 (1·7, 3·5)	92·4 (80·6, 97·0)
Panbio-ELISA	8/1387	0·3 (0·1, 0·6)	34/698	2·5 (1·7, 3·5)	88·3 (74·8, 94·6)
TELL ME FAST-RDT	3/827	0·2 (0·0, 0·5)	15/437	1·7 (1·0, 2·9)	89·3 (63·0, 96·9)
SD BIOLINE-RDT	9/1104	0·4 (0·2, 0·8)	25/560	2·3 (1·5, 3·4)	81·8 (60·9, 91·5)
OnSite-RDT	1/759	<0·1 (0·0, 0·4)	10/388	1·3 (0·6, 2·4)	94·9 (60·0, 99·3)
Participants ≥6 years					
EUROIMMUN-ELISA	8/1446	0·3 (0·1, 0·5)	40/738	2·8 (2·0, 3·7)	89·8 (78·3, 95·2)
Panbio-ELISA	11/1503	0·4 (0·2, 0·7)	42/768	2·8 (2·0, 3·8)	86·6 (74·1, 93·1)
TELL ME FAST-RDT	4/874	0·2 (0·1, 0·6)	16/461	1·8 (1·0, 2·9)	86·6 (60·0, 95·5)
SD BIOLINE-RDT	11/1186	0·5 (0·2, 0·8)	31/604	2·6 (1·8, 3·7)	81·8 (63·9, 90·9)
OnSite-RDT	2/804	0·1 (0·0, 0·4)	11/413	1·3 (0·7, 2·4)	90·6 (57·5, 97·9)
Participants <9 years*					
EUROIMMUN-ELISA	6/239	1·3 (0·5, 2·7)	14/117	6·0 (3·3, 9·9)	79·4 (46·4, 92·1)
Panbio-ELISA	4/250	0·8 (0·2, 2·0)	15/135	5·6 (3·2, 9·1)	85·9 (57·5, 95·3)
TELL ME FAST-RDT	1/108	0·5 (0·0, 2·5)	4/56	3·6 (1·0, 8·9)	87·1 (–15·2, 98·6)
SD BIOLINE-RDT	3/185	0·8 (0·2, 2·3)	10/89	5·6 (2·7, 10·1)	85·9 (48·9, 96·1)
OnSite-RDT	2/108	0·9 (0·1, 3·3)	5/54	4·6 (1·5, 10·5)	80·1 (–2·7, 96·1)

Data pooled from CYD14 and CYD15. Density incidence is presented as cases per 100 person-years; person-years at risk are the sum of years for which the participants contributed to the analysis; CI for the single proportion are calculated using the exact binomial method (Clopper-Pearson method); vaccine efficacy and associated CI are calculated using the Cox regression model.

CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; n, number of cases; N, number of test-positive participants; RDT, rapid diagnostic test; VCD, virologically-confirmed dengue

*participants from CYD14 only

Table S10. Vaccine efficacy against hospitalised VCD in test positive subjects by different age cut offs (Month 0 – Month 72)

	CYD-TDV group		Placebo group		Vaccine Efficacy (95% CI)
	n/N	Density incidence (95% CI)	n/N	Density incidence (95% CI)	
Participants ≥9 years					
EUROIMMUN-ELISA	6/1336	<0·1 (0·0, 0·2)	8/679	0·2 (0·1, 0·4)	61·0 (–12·5, 86·5)
Panbio-ELISA	7/1387	<0·1 (0·0, 0·2)	12/698	0·3 (0·2, 0·5)	70·3 (24·6, 88·3)
TELL ME FAST-RDT	0/827	0·0 (0·0, 0·1)	4/437	0·2 (0·0, 0·4)	100 (NC, 100)
SD BIOLINE-RDT	1/1104	<0·1 (0·0, 0·1)	6/560	0·2 (0·1, 0·4)	91·4 (28·2, 99·0)
OnSite-RDT	2/759	<0·1 (0·0, 0·2)	2/388	<0·1 (0·0, 0·3)	45·8 (–285·1, 92·4)
Participants ≥6 years					
EUROIMMUN-ELISA	7/1446	<0·1 (0·0, 0·2)	14/738	0·3 (0·2, 0·6)	73·7 (34·8, 89·4)
Panbio-ELISA	8/1503	<0·1 (0·0, 0·2)	19/768	0·4 (0·3, 0·7)	78·0 (49·7, 90·4)
TELL ME FAST-RDT	0/874	0·0 (0·0, 0·1)	5/461	0·2 (0·1, 0·5)	100 (NC, 100)
SD BIOLINE-RDT	2/1186	<0·1 (0·0, 0·1)	9/604	0·3 (0·1, 0·5)	88·3 (45·7, 97·5)
OnSite-RDT	2/804	<0·1 (0·0, 0·2)	4/413	0·2 (0·0, 0·4)	72·9 (–47·8, 95·0)
Participants <9 years*					
EUROIMMUN-ELISA	3/239	0·2 (0·0, 0·6)	9/117	1·3 (0·6, 2·5)	84·0 (40·7, 95·7)
Panbio-ELISA	3/250	0·2 (0·0, 0·6)	11/135	1·4 (0·7, 2·5)	85·6 (48·5, 96·0)
TELL ME FAST-RDT	1/108	0·2 (0·0, 0·9)	3/56	0·9 (0·2, 2·7)	82·9 (–64·3, 98·2)
SD BIOLINE-RDT	2/185	0·2 (0·0, 0·7)	6/89	1·2 (0·4, 2·5)	84·4 (22·7, 96·9)
OnSite-RDT	1/108	0·2 (0·0, 0·9)	4/54	1·3 (0·4, 3·3)	87·9 (–8·0, 98·7)

Data pooled from CYD14 and CYD15. Density incidence is presented as cases per 100 person-years; person-years at risk are the sum of years for which the participants contributed to the analysis; CI for the single proportion are calculated using the exact binomial method (Clopper-Pearson method); vaccine efficacy and associated CI are calculated using the Cox regression model.

CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; n, number of cases; N, number of test-positive participants; NC, not calculable; RDT, rapid diagnostic test; VCD, virologically-confirmed dengue

*participants from CYD14 only