SUPPLEMENTARY APPENDIX

Materials and Methods

Results

MATERIALS AND METHODS

Standard Operation Procedure (SOP) for the collection of salivary samples.

Each enrolled subject underwent self-collection of whole saliva samples under the supervision of a trained provider.
Briefly, the procedure took place at the same time in the morning and with the same environmental conditions of light and noise at the three time points established in the protocol.
Each subject did not eat, drink or smoke for at least one hour before the sample collection.
When performing the self-collection, each subject avoided to swallow for about one minute and allowed his/her saliva to accumulate in the floor of the mouth. Afterwards, the saliva was spitted into a sterile container and the procedure was repeated until a volume of 1.8 - 2.0 mL was collected. Immediately after the ending of the procedure, the salivary sample was transferred into a cryovial and then stored at -80 °C.

RESULTS

Table S1. Demographic characteristics and side effects after the first and second vaccine doses, in the overall sample and by previous SARS-CoV-2 exposure.

Variables	All sample	No previous SARS-CoV-2 (SN)	Previous SARS-CoV-2 (SP)	p-value
No. Of subjects	60	50	10	-
Age, years	41·2±10·4	42·6±10·8	34·4±3·6	0·55
Men, n (%)	20 (33·3%)	17 (34%)	3 (30%)	0·17
Side effects after first vaccine dose, n (%)				
Fever	4 (6·7%)	1 (2%)	3 (30%)	0·001
Headache	4 (6·7%)	4 (8%)	0 (0%)	0·35
Weakness	8 (13·3%)	5 (10%)	3 (30%)	0·09
Myalgia/Arthralgia	0 (0%)	0 (0%)	0 (0%)	-
Malaise/chills	4 (6·7%)	4 (8%)	0 (0%)	0·35
Lymphoadenopathy	0 (0%)	0 (0%)	0 (0%)	-
Nausea	0 (0%)	0 (0%)	0 (0%)	-
Dyarrhea	1 (1·7%)	1 (2%)	0 (0%)	0·65
Other systemic side effects	2 (3·3%)	2 (4%)	0 (0%)	0·52
At least one side effect	19 (31·7%)	15 (30%)	4 (40%)	0·53
Side effects after second vaccine dose, n (%)				
Fever	17 (28·3%)	13 (26%)	4 (40%)	0·37
Headache	17 (28·3%)	11 (22%)	6 (60%)	0·01
Weakness	16 (26·7%)	13 (26%)	3 (30%)	0·79
Myalgia/Arthralgia	26 (43·3%)	23 (46%)	3 (30%)	0·35
Malaise/chills	6 (10%)	5 (10%)	1 (10%)	1·00
Lymphoadenopathy	5 (8·3%)	5 (10%)	0 (0%)	0·30
Nausea	7 (11·7%)	5 (10%)	2 (20%)	0·37
Dyarrhea	3 (5%)	3 (6%)	0 (0%)	0·43
Other systemic side effects	1 (1·7%)	1 (2%)	0 (0%)	0·65
At least one side effect	44 (73·3%)	37 (74%)	7 (70%)	0·79

In the table mean ± SD for continuous variables and absolute (relative) frequencies for dichotomic variables.

Figure S1: Concentrations of S1-binding IgG antibodies (Ab) in serum samples using the CLIA assay, at baseline (T0), two weeks after the BNT162b2 priming dose (T1) and two weeks after the boost dose (T2). The horizontal and vertical solid lines correspond to the sample median and interquartile range, respectively. The dotted horizontal line at 15 AU/ml corresponds to the cut-off for positivity.

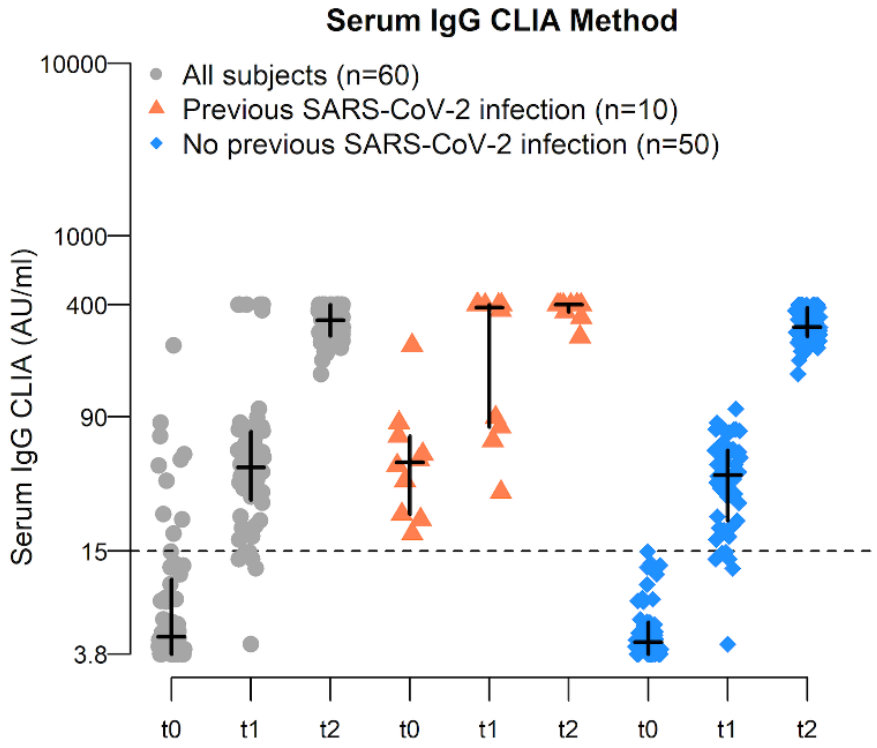

Table S2: Time trends for serum IgG Ab as measured with CLIA method, by previous SARS-CoV-2 exposure.

Variable, time	All subjects (n=60)		No previous SARS-CoV-2 (SN; n=50)		Previous SARS-CoV-2 (SP; n=10)		p-value[2]	p-value[3]
	Mean[1]	Δ (IC 95%)	Mean[1]	Δ (IC 95%)	Mean[1]	Δ (IC 95%)		
Serum IgG, CLIA Method (AU/ml)[a]								
T0	15·4	-	5·6	-	64·2	-		0·003
T1	80·0	64·6 (42·5; 86·8)	43·2	37·7 (19·0; 56·3)	263·7	199·4 (157·8; 241·1)	0·003	<·0001
T2	319·9	304·6 (287·8; 321·4)	308·7	303·1 (281·8; 324·3)	376·3	312·1 (264·5; 359·6)		0·001

a: Change in geometric mean concentration, modelled through a linear regression model for repeated measures, with unstructured variance-covariance matrix and adjusting for baseline value.
1: Geometric mean concentration
2: p-value testing homogeneity of trends between subjects with and without previous SARS-CoV-2 infection.
3: p-value testing homogeneity of geometric mean values in IgG Ab between subjects with and without previous SARS-CoV-2 infection, at each time.

Table S3: Sample characteristics by presence of salivary IgA at T2.

Variables	IgA non-responders (NR)	IgA responders (R)	p-value[1]
No. Of subjects	29	31	-
Age, years	40·1±11·3	42·3±9·6	0·41
Men, n (%)	11 (37·9%)	9 (29.0%)	0·47
SARS-CoV-2 infection at baseline, n (%)	3 (10·3%)	7 (22.6%)	0·20
Serum IgG at T2, CLIA method[a]	288·0 (261·0; 400·0)	346·0 (269·0; 400·0)	0·07
Serum IgG at T2, ELISA method[a]	18672·0 (11464·0; 56928·0)	26388·0 (5707·0; 54777·0)	0·44
Serum IgA at T2, ELISA method[a]	51·7 (31·7; 90·2)	117·0 (70·1; 155·4)	**0·005**
Serum IgA at T2 >0, n (%)	27 (93·1%)	30 (96·8%)	0·51
Salivary IgG at T2, ELISA method[a]	8·1 (2·7; 16·9)	17·9 (8·4; 37·0)	0·07

a: In the table mean ± SD for age; median (P25-P75) for IgG/IgA values; absolute (relative) frequencies for dichotomic variables.
1: p-values from t-test for age; chi-square test (1 dof) for gender, and Wilcoxon rank test for IgG and IgA variables.

Table S4: Association between serum and salivary IgG and IgA detection with the demographic characteristics of the population and reported side effects after the vaccination, overall sample.

Variables	Serum IgG (log scale)		Salivary IgG (log scale)		Serum IgA (log scale)		Salivary IgA (log scale)	
	Beta	p-value[1]	Beta	p-value[1]	Beta	p-value[1]	Beta	p-value[1]
Age, years	-0·018	0·25	0·015	0·391	-0·016	0·55	0·07	0·01
Men, n (%)	0·278	0·39	0·074	0·830	0·703	0·20	-0·27	0·62
Side effects after second vaccine dose, n (%)								
Fever	0·443	0·27	0·249	0·557	-1·521	0·02	0·40	0·55
Headache	0·183	0·62	-0·203	0·601	0·409	0·51	0·15	0·81
Weakness	0·354	0·33	0·871	0·025	-0·179	0·77	1·51	0·01
Myalgia/Arthralgia	-0·101	0·77	0·165	0·659	-0·286	0·63	-0·43	0·47
Malaise/chills	0·120	0·83	-0·408	0·480	1·695	0·07	-0·40	0·66
Lymphoadenopathy	-0·138	0·80	-0·340	0·562	1·866	0·05	0·14	0·88
Nausea	-0·906	0·07	0·513	0·327	1·433	0·08	2·74	0·00
Dyarrhea	-0·723	0·33	-0·358	0·651	0·815	0·52	-1·85	0·14

In the table mean ± SD for continuous variables and absolute (relative) frequencies for dichotomic variables.
1: p-value testing the null hypothesis of beta-coefficient equal to zero in multivariate regression model, from Wald chi-square tests.

Table S5: Association between time from COVID-19 diagnosis and symptoms with the baseline levels of both serum and salivary IgG/IgA Ab among the subjects with previous SARS-CoV-2 infection (data available from 8 subjects).

Variables	N	Serum IgG, CLIA		Serum IgG		Serum IgA		Salivary IgG		Salivary IgA	
		Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median
Time from COVID-19 diagnosis, months											
6 or less	4	50·0	46·4	39·4	2·0	0·0	0·0	0·2	0·0	0·0	0·0
More than 6	3	110·3	50·9	662·0	339·5	7·5	10·9	1·3	0·0	0·3	0·1
Symptoms											
Aymptomatic	3	39·1	38·2	1·4	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Symptomatic	5	96·7	69·5	646·4	339·5	25·9	10·9	0·9	0·0	0·2	0·0

In the table: sample mean and median, in the 8 subjects with previous SARS-CoV-2 infection and available data on diagnosis date and symptoms.

Table S6: AUC (with 95% confidence intervals) and optimal cut-off values[a] for ELISA determinations of serum IgG and salivary IgG, as compared to serum IgG quantification by CLIA.

	AUC (95%CI)	Optimal cut point		
		Value	Se (95%CI)	Sp (95%CI)
Serum IgG CLIA Method >=15 AU/ml				
Serum IgG, ELISA Method	0·979 (0·960; 0·997)	125·8	0·927 (0·866; 0·966)	1·0 (0·936; 1·0)
Salivary IgG, ELISA Method	0·789 (0·735; 0·844)	0·127	0·645 (0·554; 0·729)	0·929 (0·827; 0·980)
Serum IgG CLIA Method >=90 AU/ml				
Serum IgG, ELISA Method	0·993 (0·986; 1·0)	904·5	0·985 (0·921; 0·999)	0·919 (0·853; 0·963)
Salivary IgG, ELISA Method	0·964 (0·939; 0·989)	1·54	0·985 (0·921; 0·999)	0·884 (0·810; 0·937)

Abbreviation: AUC = Area Under the ROC curve; Se = Sensitivity; Sp = Specificity; CI = confidence interval
a: Youden criteria: criterion based on maximization of (Se+Sp-1).

Table S7: Detection of anti-RBD NAb and inhibition activity (INH) by competitive ELISA in both serum and saliva, in the overall sample and by previous SARS-CoV-2 exposure.

Time from vaccination	Overall sample (n=60)				No previous SARS-CoV-2 (n=50)				Previous SARS-CoV-2 (N=10)			
	Serum NAb		*Salivary NAb*		*Serum NAb*		*Salivary NAb*		*Serum NAb*		*Salivary NAb*	
	N[1] (%)	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**
Baseline (T0)	7 (11·7)	60·9 (23·5)	0 (0)	_	0 (0)	_	0 (0)	_	7 (70·0)	60·9 (23·5)	0 (0)	_
Two weeks after the first dose (T1)	35 (58·3)	60·1 (21·3)	8 (13·3)	49·4 (16·8)	25 (50·0)	51·9 (16·7)	2 (4·0)	38·3 (1·9)	10 (100·0)	80·6 (17·1)	6 (60·0)	53·2 (18·1)
Two weeks after the second dose (T2)	60 (100·0)	91·5 (1·1)	15 (25)	56·4 (14·6)	50 (100·0)	91·4 (1·1)	9 (18·0)	52·2 (14·5)	10 (100·0)	92·2 (0·7)	6 (60·0)	62·8 (13·2)

1: Number of subjects with the presence of NAb in the sample, and prevalence in the overall sample expressed as percentage.
2: Mean inhibitory activity detected in the subjects with NAb in the sample, and standard deviation.

Table S8: Detection of anti-wildtype and anti-Delta RBD NAb and inhibitory activity (INH) by competitive ELISA in both serum and saliva at T2, in the 15 subjects who showed INH against the wild-type antigen and by previous SARS-CoV-2 exposure.

RBD antigen	Overall sample (n=15)				No previous SARS-CoV-2 (n=9)				Previous SARS-CoV-2 (N=6)			
	Serum NAb		*Salivary NAb*		*Serum NAb*		*Salivary NAb*		*Serum NAb*		*Salivary NAb*	
	N[1] (%)	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**	**N[1] (%)**	**INH[2] (SD)**	**N[1] (%)**	**INH[2](SD)**	**N[1] (%)**	**INH[2] (SD)**
Wild-type RBD (Wuhan-Hu-1), T2	15 (100·0)	91·5 (1·1)	15 (100)	56·4 (14·6)	9 (60·0)	91·4 (1·1)	9 (60·0)	52·2 (14·5)	6 (40·0)	92·2 (0·7)	6 (40·0)	62·8 (13·2)
Delta variant RBD (B.617.2; E484Q, L452R), T2	15 (100·0)	89·5 (1·8)	15 (100)	53·5 (15·7)	9 (60·0)	88·7 (2·0)	9 (60·0)	48·9 (17·8)	6 (40·0)	90·6 (0·3)	6 (40·0)	60·3 (9·3)

1: Number of subjects with the presence of NAb in the sample, and prevalence in the overall sample expressed as percentage.
2: Mean inhibitory activity detected in the subjects with NAb in the sample, and standard deviation.

Table S9: Sample characteristics by presence of neutralizing antibodies in saliva at T2.

Variables	Salivary NAb-negative (T2)	Salivary NAb-positive (T2)	p-value[1]
No. Of subjects	45	15	-
Age, years	41·5±10·5	40·3±10·6	0·69
Men, n (%)	15 (33·3%)	5 (33·3%)	1·00
SARS-CoV-2 infection at baseline, n (%)	4 (8·9%)	6 (40·0%)	0·005
Serum IgG at T2, CLIA method[a]	296·0 (263·0; 355·0)	400·0 (324·0; 400·0)	**0·04**
Serum IgG at T2, ELISA method[a]	14676·0 (7325·0; 45213·0)	51592·0 (23041·0; 74614·0)	**0·01**
Serum IgA at T2, ELISA method[a]	82·4 (48·0; 123·3)	84·0 (27·9; 155·4)	0·77
Salivary IgG at T2, ELISA method[a]	8·3 (3·6; 13·7)	46·6 (23·1; 75·0)	**<·0001**
Salivary IgA at T2, ELISA method[a]	0·0 (0·0; 0·34)	0·28 (0·0; 1·3)	**0·04**

a: In the table mean ± SD for age; median (P-25-P75) for IgG/IgA values; absolute (relative) frequencies for dichotomic variables.
1: p-values from t-test for age; chi-square test (1 dof) for gender; and Wilcoxon rank test for IgG and IgA variables.