Supplementary Materials

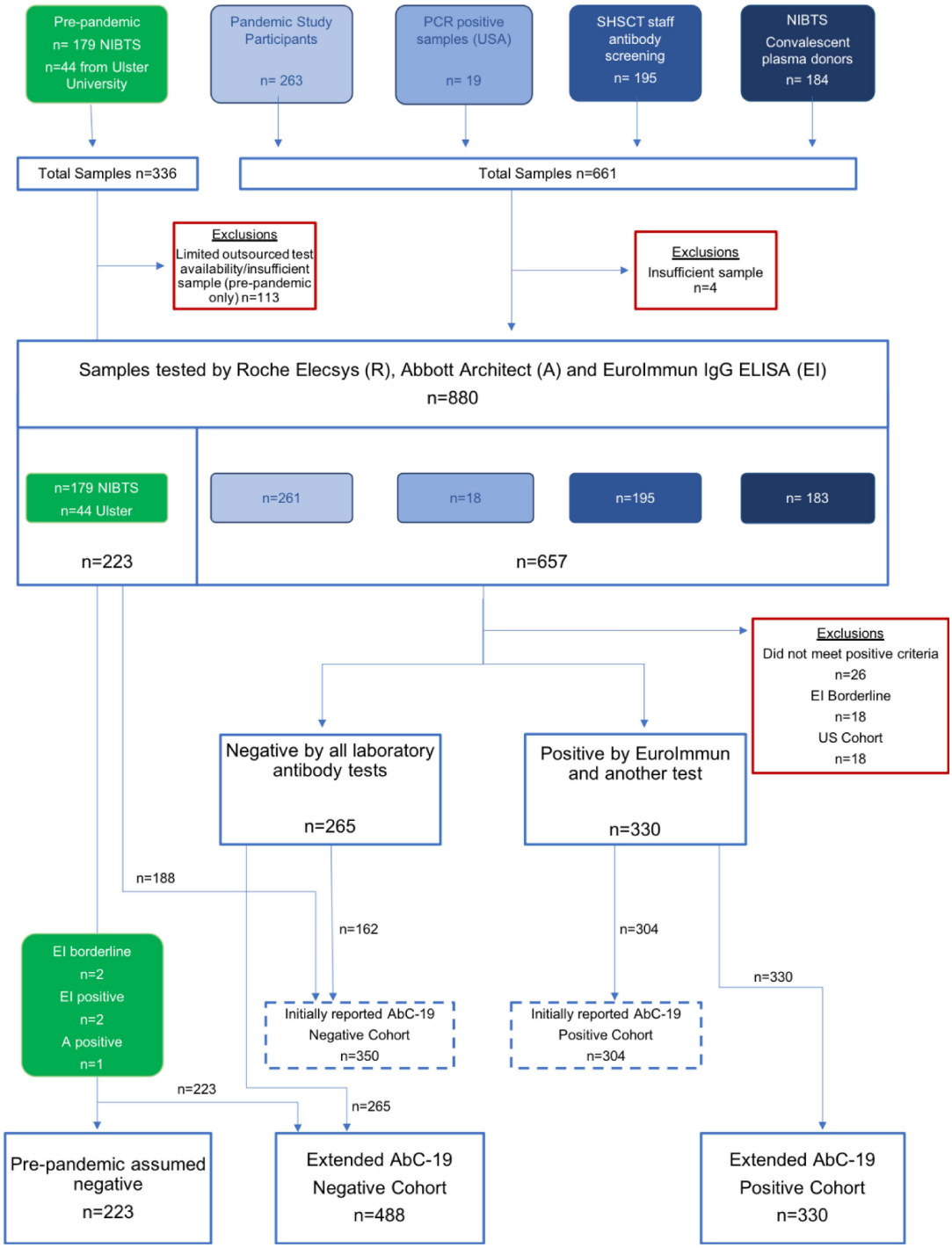
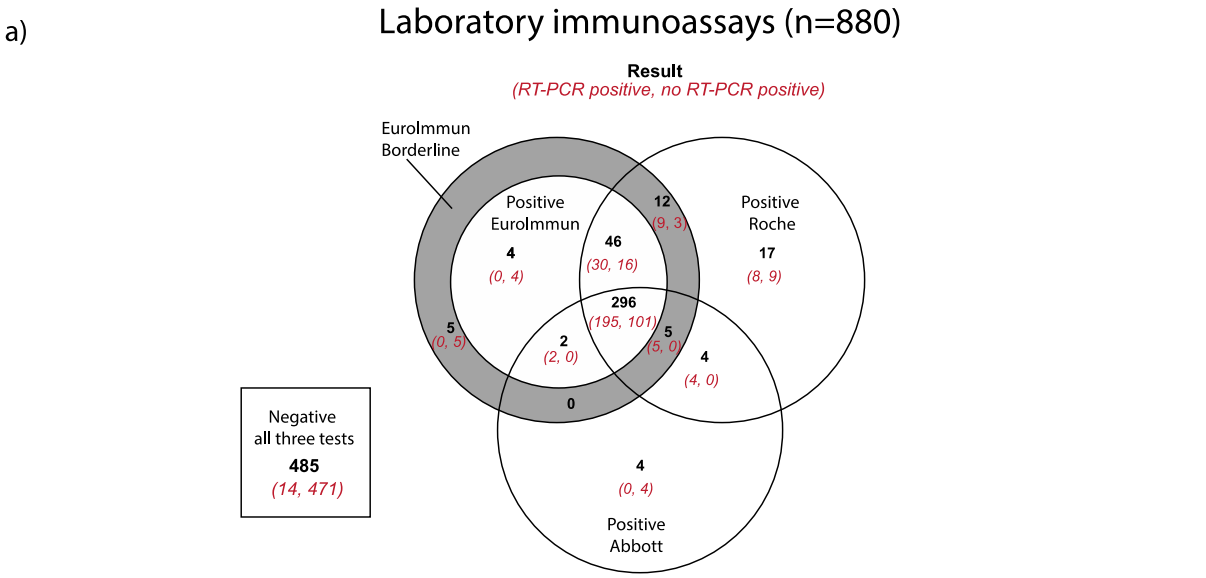


Figure S1: Flow of participant plasma samples through cross-sectional study.

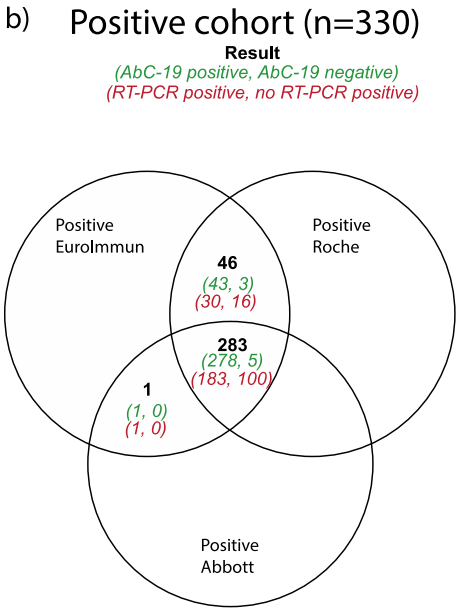
All available samples from participants within each cohort, and the included and excluded samples at all stages. Freeze thaw cycles were closely monitored for all sample aliquots. Pre-pandemic samples taken forward for Roche, Abbott and EuroImmun testing were selected based on aliquot volume and availability.

Figure S2: Visual Score card for quantitative interpretation of AbC-19 LFIA test bands. A scale of 0 (not pictured, negative-no test line visible) to 10 (positive-strongest test line). Any LFIA scoring 1 or above was classified as positive.

a)


Laboratory immunoassays (n=880)
Result
(RT-PCR positive, no RT-PCR positive)
EuroImmun Borderline
Positive EuroImmun
Positive Roche
Positive Abbott
12 (9, 3)
4 (0, 4)
46 (30, 16)
17 (8, 9)
296 (195, 101)
5 (0, 5)
2 (2, 0)
5 (5, 0)
4 (4, 0)
0
4 (0, 4)
Negative all three tests
485 (14, 471)

b)


Positive cohort (n=330)
Result
(AbC-19 positive, AbC-19 negative)
(RT-PCR positive, no RT-PCR positive)
Positive EuroImmun
Positive Roche
Positive Abbott
46 (43, 3) (30, 16)
283 (278, 5) (183, 100)
1 (1, 0) (1, 0)

c)

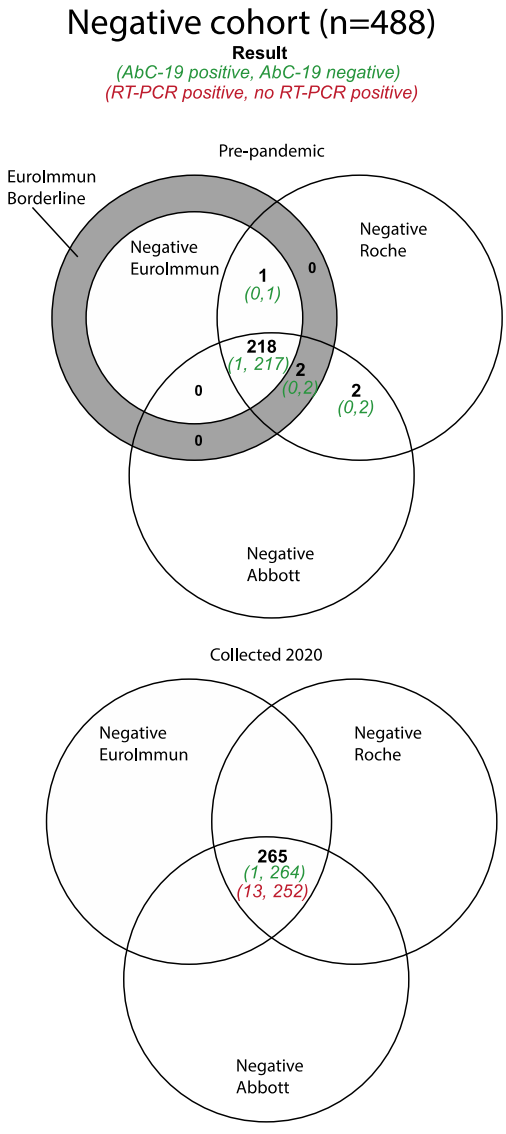

Negative cohort (n=488)
Result
(AbC-19 positive, AbC-19 negative)
(RT-PCR positive, no RT-PCR positive)
Pre-pandemic
EuroImmun Borderline
Negative EuroImmun
Negative Roche
Negative Abbott
1 (0,1)
0
218 (1, 217)
2 (0,2)
0
2 (0,2)
0
Collected 2020
Negative EuroImmun
Negative Roche
Negative Abbott
265 (1, 264) (13, 252)

Figure S3: Venn diagrams demonstrating result overlap between laboratory assays in a) the initial immunoassay cohort (n=880), b) the positive and c) negative cohorts assessed with AbC-19 TT3. Result in each circle overlap in bold, (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below. Where AbC-19 was analysed, (AbC-19 positive, AbC-19 negative) denoted in green.

Robertson LJ, *et al. BMJ Open* 2021; 11:e048142. doi: 10.1136/bmjopen-2020-048142

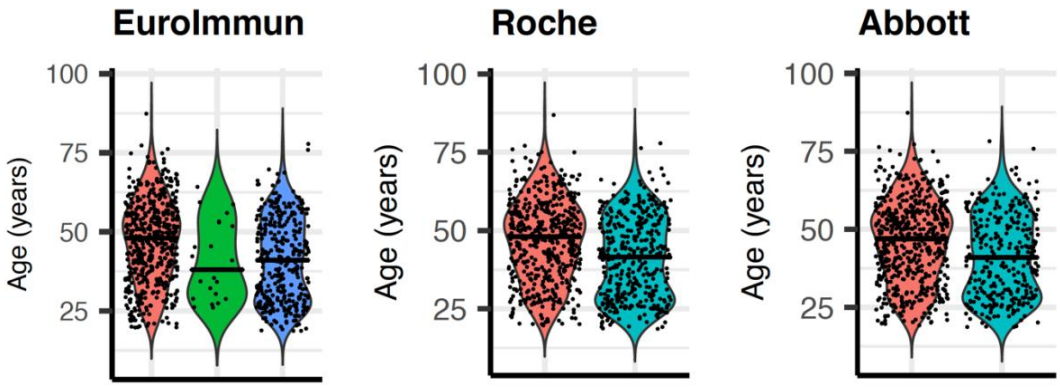


Figure S4: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.

The above graphs allow comparison of the distributions and probability density of ages for EuroImmun, Roche and Abbott immunoassays. Wider areas of the violin plot represent high probability density, whilst narrow areas represent low probability density. Horizontal bar indicates median age. The red violin plots represent the negative results, the green violin plot represent the borderline results and the blue/turquoise violin plots represent the positive results.

Robertson LJ, *et al. BMJ Open* 2021; 11:e048142. doi: 10.1136/bmjopen-2020-048142

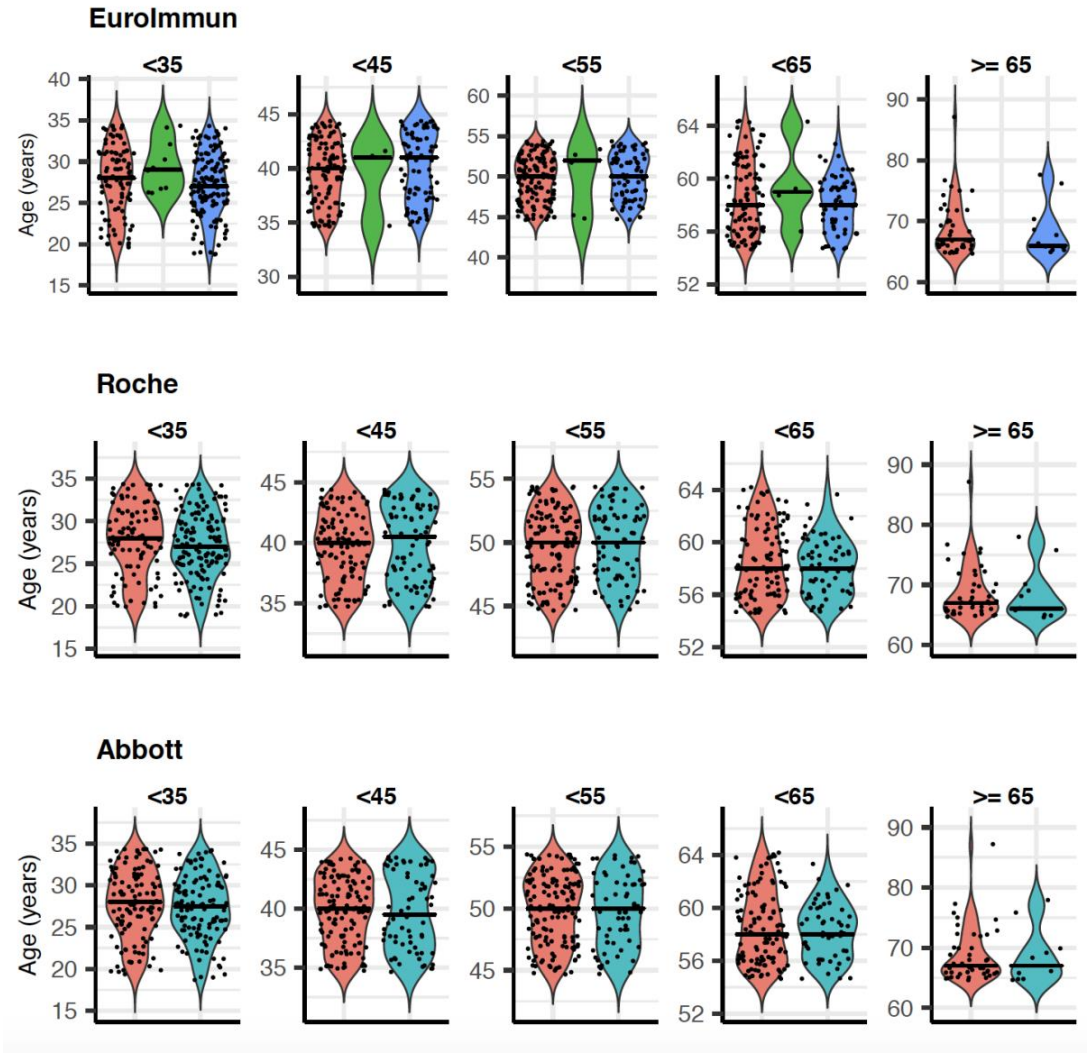


Figure S5: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.

The above figure presents graphs for each immunoassay (EuroImmun, Roche and Abbott) with the corresponding age groups <35 years, <45 years, <55 years, <65 years and >= 65 years. The red violin plots represent the negative results, the green violin plot represents the borderline EuroImmun results, and the blue/turquoise violin plots represent the positive results (n=848).

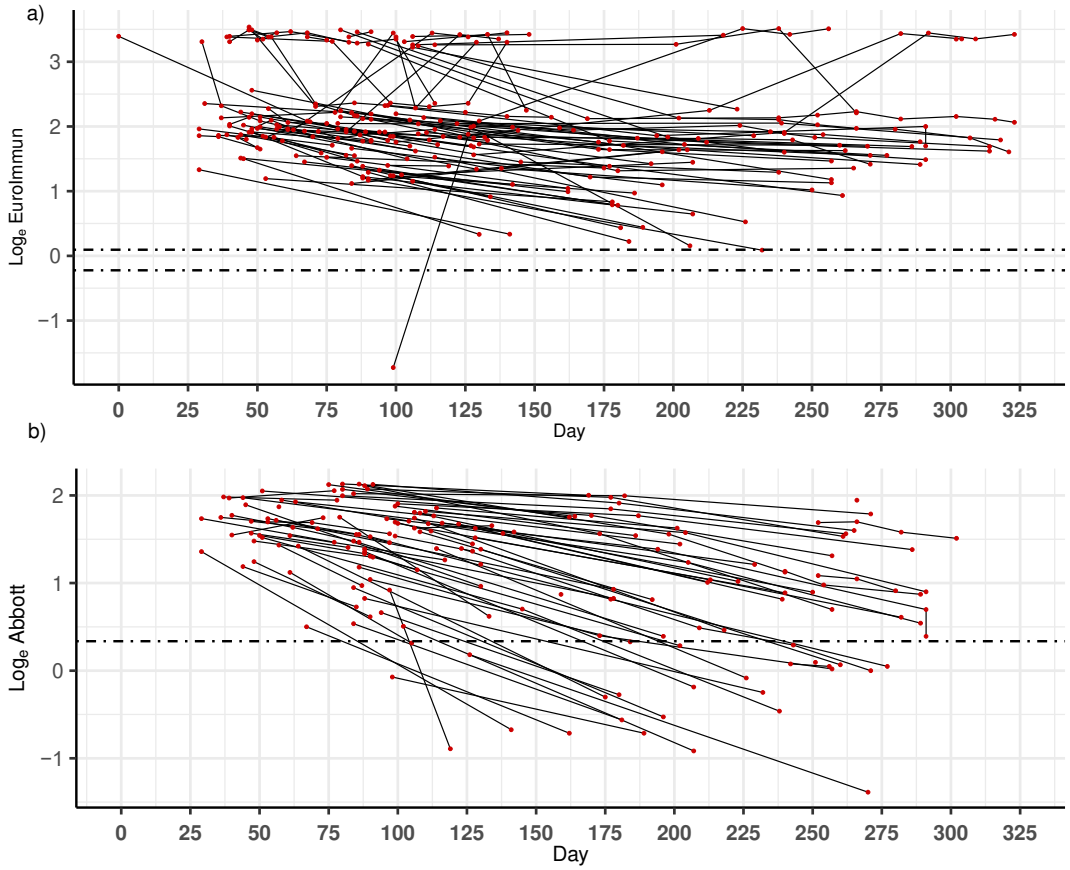


Figure S6: Longitudinal analysis of convalescent plasma donor sequential samples (2-9 samples per individual) by a) EuroImmun ELISA or b) Abbott immunoassay. a) n=101 individuals, grey shading indicates borderline region, upper dotted line indicates positivity threshold (1.1), lower dotted line indicates negativity threshold (0.8) b) n=75 individuals, dotted line indicates positivity threshold (1.4). Dots represent log-transformed quantitative values for each sample, lines connect samples from the same individual.

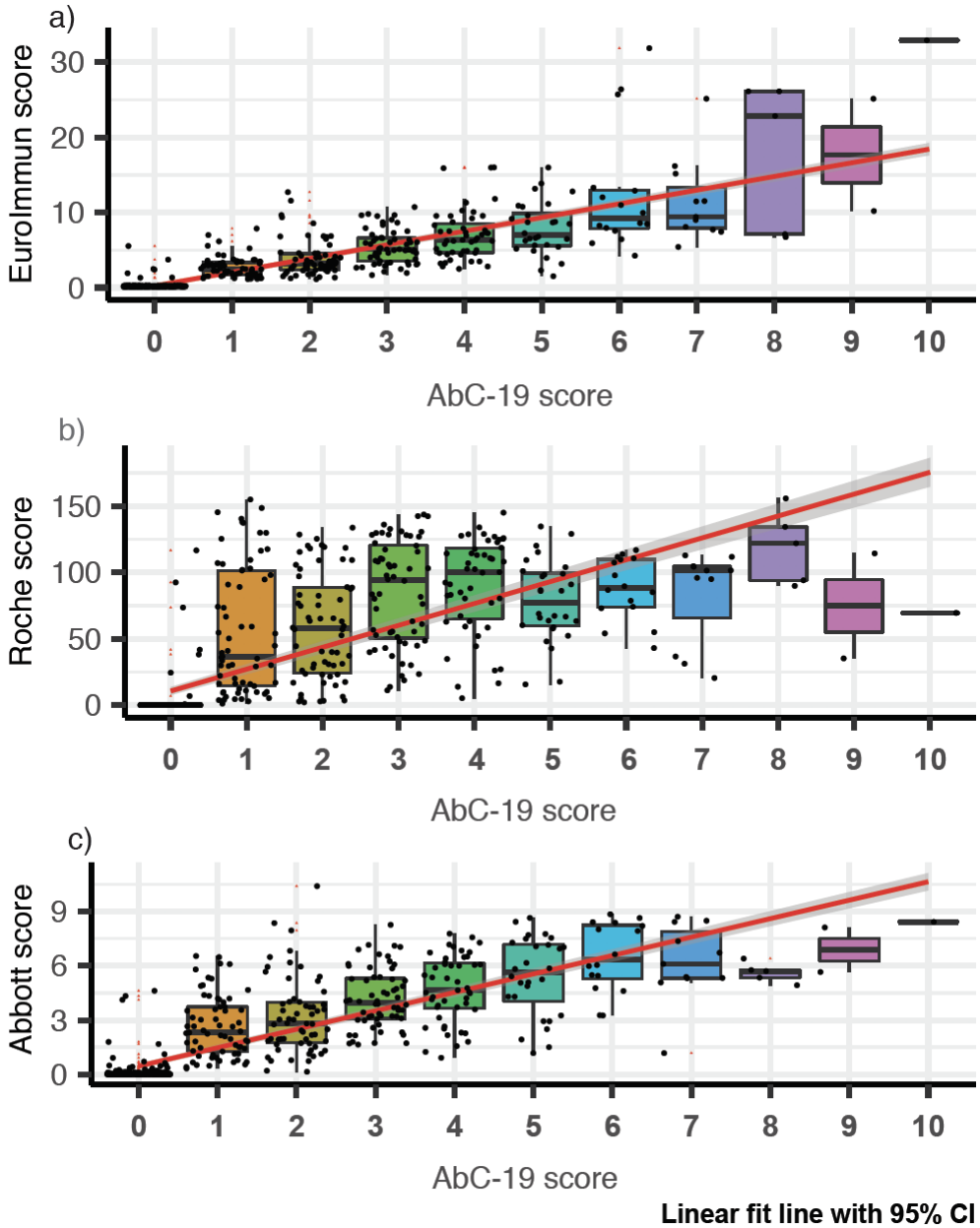


Figure S7: AbC-19 initially reported cohort n=654 correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing TT3 AbC-19 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line of best fit with 95% confidence interval shaded in grey. Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).

Robertson LJ, *et al. BMJ Open* 2021; 11:e048142. doi: 10.1136/bmjopen-2020-048142

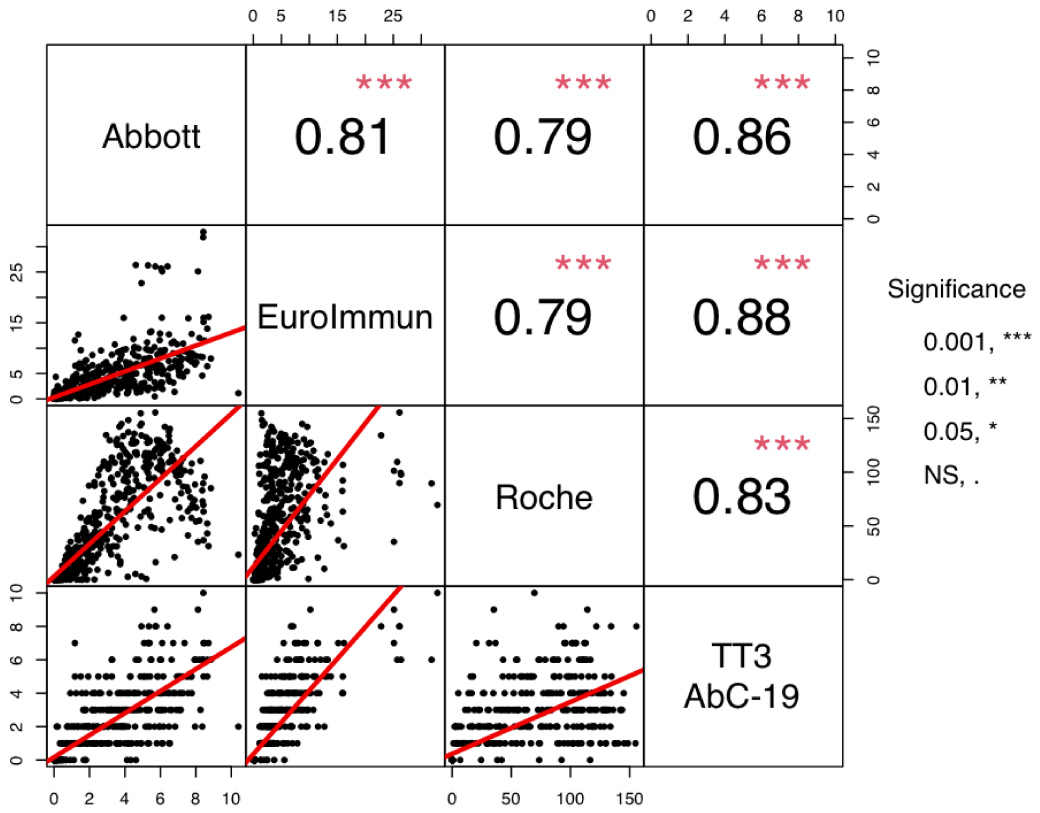


Figure S8: **Correlation matrix between Abbott, EuroImmun, Roche and initially reported AbC-19 cohort (n=654) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly correlated $p<0.001$.

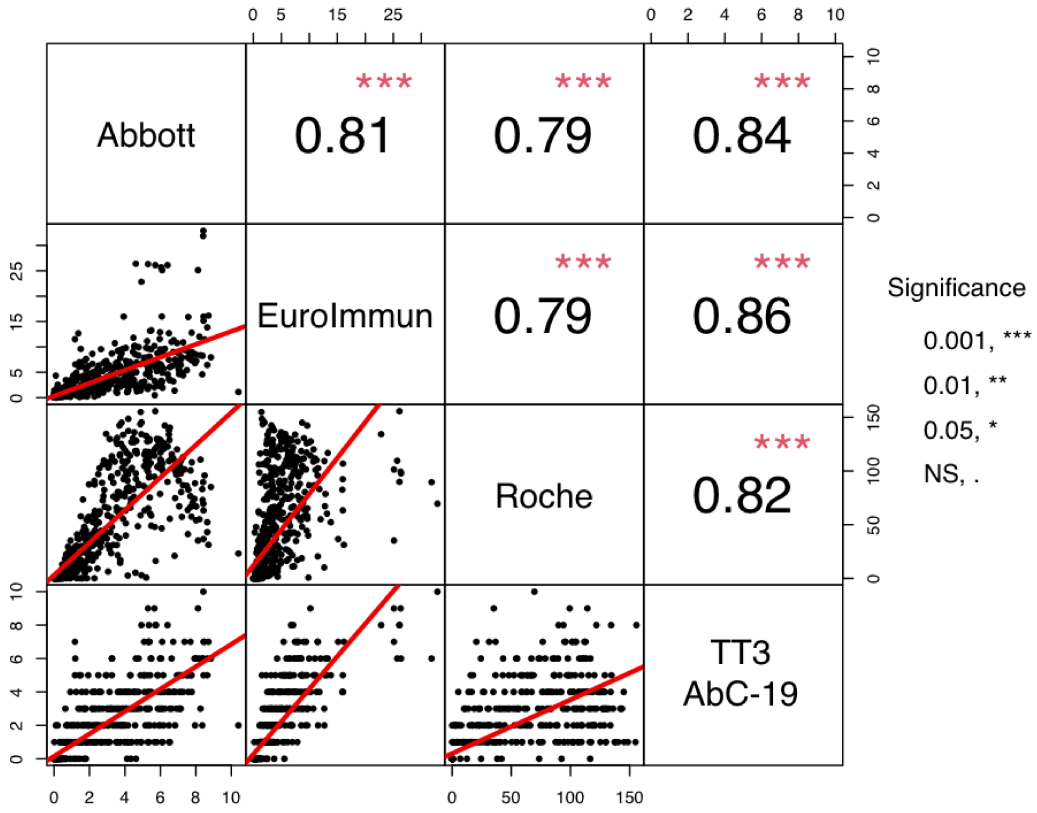


Figure S9: **Correlation matrix between Abbott, EuroImmun, Roche and extended AbC-19 cohort (n=818) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly correlated $p<0.001$.

Robertson LJ, *et al. BMJ Open* 2021; 11:e048142. doi: 10.1136/bmjopen-2020-048142

Figure S10: NIBSC external reference serology standards and known respiratory virus serology samples.

The scorecard score 0-10 was annotated on test cassette beneath sample ID when agreed by three independent experienced researchers. All LFIAs had a visible control line.

Table S1: Summary specifications for SARS-CoV-2 immunoassays investigated.

Immunoassay	Principle	Antigen Target	Assay Time (min)	Antibody Detected	Measurement	Result	Calibration	Evaluation of results	Results
EuroImmun ELISA	Enzyme-linked immunosorbent assay (enzyme-HRP)	S1 domain of the spike protein	120	IgG	Photometric measurement of the color intensity using wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm	OD (Optical density)	One Positive calibrator	OD of clinical sample/OD of calibrator	< 0.8 Negative, ≥ 0.8 to <1.1 Borderline, ≥ 1.1 Positive
Roche Elecsys immunoassay	Electro-chemiluminescence	Nucleocapsid	18	IgG, IgA and IgM	Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier	RLU (Relative Light Intensity)	One Positive calibrator and one Negative calibrator	The analyzer automatically calculates the cut-off based on the measurement of ACOV2 Cal1 (negative) and ACOV2 Cal2 (positive). The result of a sample is given either as reactive or non-reactive as well as in the form of a cut-off index (COI; signal sample/cut-off).	< 1.0 Negative, ≥ 1.0 Positive
Abbott Architect SARS-CoV-2	Chemiluminescent microparticle immunoassay	Nucleocapsid	30	IgG	The resulting chemiluminescent reaction is measured as a relative light unit (RLU).	RLU (Relative Light Intensity)	One Positive calibrator	Results are reported by dividing the sample result by the calibrator result (mean of 3 calibrators). The default result unit for the SARS-CoV-2 IgG assay is Index (S/C).	< 1.4 Negative, ≥ 1.4 Positive
TT3 AbC-19	Rapid Point of Care Lateral Flow Immunoassay	Full length Spike protein	20	IgG	The colour intensity of the test line is analysed using the reference score card.	Binary	The presence of a control line indicates the test is valid.	A result is positive if there is both a test line and a control line, whilst a result is negative if only the control line is present.	Using the reference score card; Positive scores ≥1 Negative scores=0

Table S2: Pandemic participant laboratory-based SARS-CoV-2 antibody result.

Breakdown of individual immunoassay results or result by one or more test.

Test	Positive (%)	Borderline (%)	Negative (%)
Abbott	310/657 (47.2%)	n/a	347/657 (62.8%)
EuroImmun	346/657 (52.7%)	20/657 (3.2%)	291/657 (44.4%)
Roche	380/657 (57.8%)	n/a	277/657 (42.2%)
One or more test	385/657 (58.6%)	3/657 (0.45%)	269/657 (40.9%)

Table S3: Positive RT-PCR samples sensitivity analysis on the AbC-19 LFIA.

RT-PCR Positive	True Positive	False Negative	Sensitivity % (95 CI)
227	209	18	92.07% (87.76%- 95.23%)
Negative by EI, R and A	**Negative by EI, R and A**	**Negative by EI, R and A**	
13	1	12	

Table S4: Analytical specificity analysis on the AbC-19 LFIA LFIAs were assessed using 34 serum samples with known other respiratory viruses, negative results for all suggests analytical specificity for SARS_CoV_2 IgG.

SAMPLE	Number of samples	Number of AbC-19 Positive results	Number of AbC-19 Negative results
H5N1 Influenza (NIBSC 7/150)	1	0	1
RSV (NIBSC 16/284)	1	0	1
Influenza B (NIBSC 9/222)	1	0	1
Bordetella Pertussis (NIBSC 89/530)	1	0	1
Influenza A	5	0	5

Influenza B	5	0	5
Respiratory syncytial virus	5	0	5
Haemophilus Influenzae	5	0	5
Seasonal coronavirus NL63	5	0	5
Seasonal coronavirus 229E	5	0	5

Table S5: AbC-19 LFIA results with NIBSC external reference samples

NIBSC standard serology samples were provided with a data sheet indicating the SARS-CoV-2 antibody levels. We measured SARS-CoV-2 antibody levels in these samples and obtained similar results with the EuroImmun IgG ELISA in our laboratory.

NIBSC #	**AbC-19 LFIA result**	**Ulster University lab result**	**NIBSC provided antibody data**				
		EuroImmun IgG (S1 domain)	**EuroImmun IgG (S1 domain)**	**EuroImmun IgA**	**In-house IgG S1**	**In-house IgG N**	**In-house IgG sSpike**
20/120	pos (10)	pos (8.39)	pos (8.59)	pos (10.1)	5580	3417	2693
20/122	pos (7)	pos (3.49)	pos (3.47)	pos (1.1)	3202	2425	1488
20/124	pos (1)	pos (1.56)	pos (1.62)	pos (1.84)	1636	3296	118
20/126	pos (1)	neg (0.60)	neg (0.64)	pos (1.63)	1181	995	8
20/128	neg (0)	neg (0.23)	neg (0.21)	neg (0.02)	<50	<50	<50
20/130	pos (8)	pos (6.96)	pos (7.77)	pos (9.74)	5388	17197	2707

Supplementary Methods

Laboratory-based immunoassays

Researchers were blinded to other test results when processing these assays.

EuroImmun Anti-SARS-CoV-2 ELISA-IgG (EuroImmun, EI 2606-9601 G) was carried out according to manufacturer's instructions. Optical density (OD) at 450nm and reference OD at 620nm was read on BMG Labtech Fluostar Omega spectrophotometer (BMG Labtech). Ratios were calculated by dividing absorbance of the clinical sample by the absorbance of EuroImmun calibrator, with a score of < 0.8 determined negative, ≥ 0.8 to <1.1 borderline and ≥ 1.1 positive. For samples provided by NIBTS, EuroImmun IgG assay data was provided to researchers.

Roche Elecsys immunoassay (Roche Diagnostics, kit 09203079190) was carried out according to manufacturer's instructions on the Roche cobas e601 (C6000 line) or e801 (C8000 line) analysers. The analyser automatically calculates the cut-off based on the measurement of ACOV2 Cal1 (negative) and ACOV2 Cal2 (positive). The result of a sample is given either as reactive or non-reactive as well as in the form of a cut-off index (COI; signal sample/cut-off). A score of <1.0 is determined negative, while a score ≥ 1.0 is positive.

Abbott Architect SARS-CoV-2 immunoassay was carried out according to manufacturer's instructions on the Abbott Architect i2000SR analyser (Abbott, kit 18115FN00, calibrator kit 17412FN00, Control kit 17531FN00). The external control is entered into a Quality Monitor programme and must be within 3 standard deviations of the mean (cumulative; External control NIBSC QCRSARSCoV-2QC1 Lot

20/B764-01). Results are reported by dividing the sample result by the calibrator result. The result unit for the SARS-CoV-2 IgG assay is Index (Sample/Calibrator). A ratio of < 1.4 is determined negative and ≥ 1.4 is determined positive.

Analytical specificity and sensitivity assessment

Four virology samples (H5N1 influenza serology 7/150, RSV serology 16/284, Influenza B 9/222 and Bordetella Pertusis 89/530) were obtained from NIBSC (National Institute for Biological Standards, Herts, UK). An additional 30 serology samples from known virus infections were a kind gift from SugenTech, Soeul, Korea. 15 of these virology samples were obtained from Trina (Trina Bioreactives AG, Switzerland) from 5 different individuals per virus (Influenza A IgG, Influenza B IgG and RSV IgG). A further 15 of these virology samples were obtained from AbBaltris, Kent from 5 different individuals per virus (Haemophilus Influenza IgG, Seasonal Coronavirus NL63 and 229E Seasonal Coronavirus). All these serology samples alongside a panel of 6 external standard research reagents (Table S4; NIBSC; Cat: 20/118 and 20/130) were assessed on the TT3 AbC-19 LFIA to confirm analytical specificity and sensitivity.