Evaluation of antibody testing for SARS-CoV-2 using ELISA and lateral flow immunoassays: Supplementary material

Supplementary methods

Pre-pandemic negative control samples

142 plasma samples designated seronegative for SARS-CoV-2 stored in aliquots at -80℃ were collected from adults (≥18 years) in the UK before December 2019 (Table S1) from three ethically approved sources:

(i) Healthy blood donors, n=60: plasma was provided by the blood bank from healthy adult donors. National Health Service Blood and Transplant (NHSBT) has donor consent for plasma to be used for research purposes if not used clinically (study numbers prefixed BD).

(ii) Organ donor samples, n=50: plasma was collected from intensive care patients who subsequently became organ donors by the UK National QUality in Organ Donation (QUOD) study (https://quod.org.uk). Ethical approval is through an NIHR Biobank REC agreement (REC 13/NW/0017; IRAS 87824) (study numbers prefixed Q).

(iii) BERT study, n=32: plasma stored from healthy volunteers for the 'BERT' study (A Study Exploring Whooping Cough Protection in Children and Adults; ClinicalTrials.gov Identifier NCT03697798)[1], an interventional, longitudinal, open label study on acellular pertussis (aP) vaccine. A single plasma sample from 32 individuals aged 20-70 years was used from various timepoints following booster with aP (study numbers prefixed AP).

Positive samples from RT-PCR confirmed COVID

Forty plasma samples were collected from adults testing positive for SARS-CoV-2 by RT-PCR from an upper respiratory tract (nose/throat) swab collected into viral transport media. Samples were processed through a nationally approved assay (targeting the RdRp gene) in accredited clinical laboratories in Colindale (Public Health England Respiratory Virus Unit), Oxford or Southampton, UK or the National Reference Center for Respiratory Viruses (Hospices Civils de Lyon), France (Suppl Table 2). Participants provided their written informed consent for recruitment into the International Severe Acute Respiratory and

Emerging Infection Consortium ('ISARIC', https://isaric.tghn.org/) study approved by the South Central - Oxford C Research Ethics Committee in England (Ref: 13/SC/0149), and by the Scotland A Research Ethics Committee in Scotland (Ref: 20/SS/0028).

Samples were obtained from three sources:

(i) Acutely sampled patients, n=16: plasma from adults admitted to Oxford University Hospitals NHS Foundation Trust, collected 3-5 days into admission at a median of 10 days (range 4-27 days) from first onset of symptoms. Cases were classified as mild (n=3), severe (n=4) and critical (n=9) based on WHO criteria[2] (study numbers numbers prefixed UKCOV-).

(ii) Healthcare workers, n=6: plasma from healthcare workers collected after a confirmed diagnosis of SARS-CoV-2 by RT-PCR, >7 days from onset of symptoms, with sufficient resolution of the clinical syndrome to allow return to work. All were clinically classified as mild disease. Samples were collected a median of 13 days after first symptoms (range 8-19 days) (study numbers prefixed HCW-).

(iii) Convalescent patients, n=18: plasma from adults recruited >28 days following first onset of symptoms and/or date of positive throat swab. All except one individual had reported mild symptoms compatible with SARS-CoV-2 infection (COVID-19); the asymptomatic individual was screened with a throat swab, having been part of an epidemiological cluster of infections during the early phase of containment, through enhanced contact tracing by Public Health England. Samples were collected a median 48 days (range 31-62 days) after first onset of symptoms and/or date of positive throat swab (study numbers prefixed COV19-).

We obtained the onset of symptoms from all participants. For acute participants this was obtained from the admission medical records. For healthcare workers and convalescent samples this was obtained during a structured interview with the participant at the time of plasma sampling, supported by available medical records. Relevant symptoms included fever, cough, malaise, myalgia, headache and/or anosmia.

Sample processing

Blood donor plasma was collected in sodium citrate. Frozen plasma bags were thawed once and then stored in 500ul aliquots at -80°C. Blood obtained from clinical COVID cases, the

BERT cohort, organ donors and healthcare workers was collected in EDTA tubes, centrifuged and plasma stored in 500ul aliquots at -80°C. Standardised procedures were used for collection and processing of plasma samples to minimise freeze/thaw cycles. For assays, samples were thawed at room temperature on the bench.

Statistical analysis

The association between ELISA results and factors including time since symptom onset, severity, need for hospital admission and age was estimated using multivariable linear regression, without variable selection. Non-linearity in relationships with continuous factors was included via natural cubic splines, choosing linear or non-linear terms and the number of knots (up to 3) based on optimal model fit assessed using the Akaike information criterion. Pairwise interaction terms between all main effects with $p<0.1$ were investigated and retained where interaction $p<0.05$.

Mixed effects logistic regression models were used to test for differences between LFIA devices allowing for each device to be tested on overlapping sets of samples (Stata "melogit"). Two models were fitted, one for samples designated SARS-CoV-2 positive, to compare sensitivity and another for samples designated SARS-CoV-2 negative to compare specificity. The fitted outcome was the test result (negative/positive), with the different devices as fixed effects and sample identifiers as a random effect. Differences between devices were determined using Stata "pwcompare" and compared with Benjamini-Hochberg corrected p-value thresholds (9 devices, so 36 pairwise tests).

Supplementary figures

Figure S1. Sensitivity and specificity of lateral flow devices compared with RT-PCR confirmed cases and pre-pandemic controls (panels A and B) and compared with ELISA results (panels C and D). The dashed lines show the 95% confidence intervals.

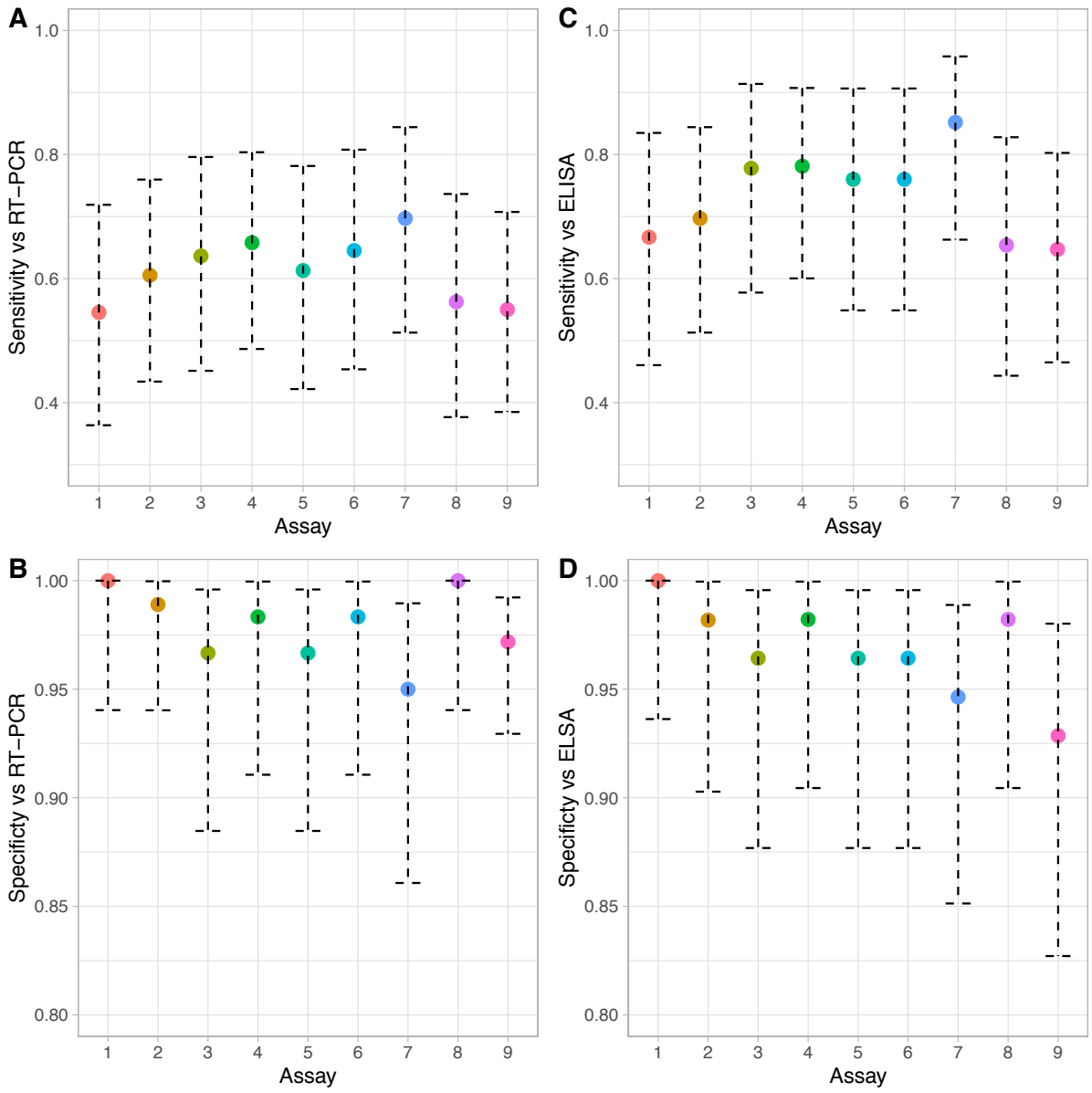
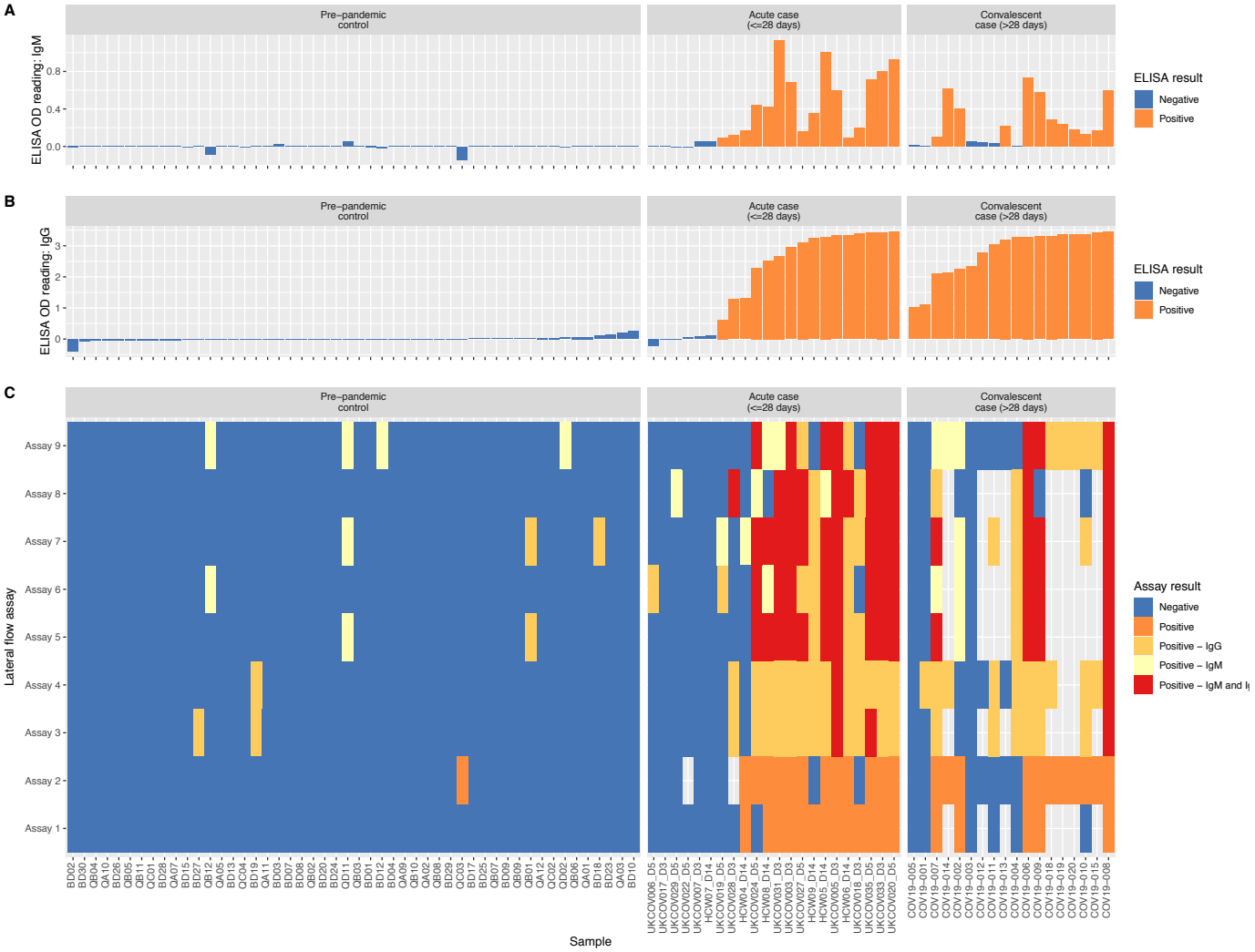


Figure S2: Comparison between ELISA and LFIA for SARS-CoV-2 designated negative and positive plasma. Quantitative optical density readout from ELISA for designated negative plasma (n=50) and from individuals with RT-PCR confirmed infection (n=40, divided into acute and convalescent plasma), for IgM (A) and IgG (B) (reproduced from main Figure 3 (A)). (C) Results from LFIA produced by nine manufacturers. Blue bars show negative test. Yellow, orange and red show positives, according to IgG, IgM, both IgG and IgM or total antibody. Grey blocks indicate missing data as a result of insufficient devices to test all samples and one assay on one device with an invalid result. Samples in all panels are ranked from left to right by quantitation of IgG (as indicated in panel B).



Supplementary Tables

Supplementary table S1. Metadata describing origin and characteristics of designated negative controls and individuals with confirmed SARS-CoV-2 infection. The negative controls represent samples collected prior to December 2019, prior to SARS-CoV-2 circulation in the UK population. The positive samples were collected from March 2020 onwards, from individuals with a confirmed diagnosis of SARS-CoV-2 infection based on RT-PCR from a nose or throat swab.

Provided as separate supplementary_table_s1.xlsx file, with separate tabs for each study cohort.

Supplementary table S2. Summary grid presenting the number of samples from each cohort tested using different assay platforms.

	SARS-COV-2 negative cohort				SARS-COV-2 positive cohort			
	Blood donors	QUOD	BERT	Total	Acute	Convales-cent	Health-care workers	Total
Assay 1	30	30	0	60	16	11	6	33
Assay 2	30	30	32	92	14	18	6	38
Assay 3	30	30	0	60	16	11	6	33
Assay 4	30	30	0	60	16	16	6	38
Assay 5	30	30	0	60	16	9	6	31
Assay 6	30	30	0	60	16	9	6	31
Assay 7	30	30	0	60	16	11	6	33
Assay 8	30	30	0	60	16	10	6	32
Assay 9	60	50	32	142	16	18	6	40
ELISA	23	27	0	50	16	18	6	40

Supplementary table S3. Multivariable regression models for relationship between ELISA IgM and IgG readings and covariates in RT-PCR positive cases.

IgG Model

Variable	Coefficient	95% Confidence interval	p-value
Age, per 10 years	-0.10	(-0.36, 0.17)	0.46
Severity, Mild	0.00		
Severity, Asymptomatic	-1.50	(-3.57, 0.56)	0.15
Severity, Severe	0.13	(-1.59, 1.86)	0.88
Severity, Critical	-1.24	(-2.81, 0.32)	0.11
Hospital Stay	1.39	(-0.63, 3.41)	0.17
Days from symptom onset, day 10	1.42	(0.47, 2.37)	
Days from symptom onset, day 20	3.47	(2.62, 4.31)	
Days from symptom onset, day 30	3.68	(3.04, 4.33)	
Days from symptom onset, day 50	2.69	(2.16, 3.23)	

Days from symptom onset fitted as non-linear term, using ns(x, df=3) term in R. Coefficients for days from symptom onset are shown having set Severity=Mild and Hospital Stay=No.

IgM Model

Variable	Coefficient	95% Confidence interval	p-value
Days from symptoms onset, per 10 days	0.02	(-0.07, 0.10)	0.67
Age, per 10 years	-0.04	(-0.12, 0.05)	0.38
Severity, Mild	0.00		
Severity, Asymptomatic	-0.30	(-1.01, 0.41)	0.40
Severity, Severe	-0.19	(-0.78, 0.4)	0.52
Severity, Critical	-0.22	(-0.73, 0.3)	0.40
Hospital Stay	0.36	(-0.27, 0.99)	0.25

Supplementary Table S4. Results of nine lateral flow immunoassays (LFIA) devices and an ELISA assay, tested with plasma classified as positive (RT-PCR positive) obtained from patients ≥10 days after onset of symptoms. Any LFIA positive result (IgM, IgG or both) was considered positive. ELISA positive samples were all positive for IgG, no sample was IgM-positive and IgG-negative.

Assay	Positive samples ≥10 days from symptom onset tested	True positives	False negative	Sensitivity (95% CI)
ELISA	31	31	0	1.00 (0.89-1.00)
1	24	16	8	0.67 (0.45, 0.84)
2	31	21	10	0.68 (0.49, 0.83)
3	24	18	6	0.75 (0.53, 0.90)
4	29	22	7	0.76 (0.56, 0.90)
5	22	17	5	0.77 (0.55, 0.92)
6	22	17	5	0.77 (0.55, 0.92)
7	24	21	3	0.88 (0.68, 0.97)
8	23	14	9	0.61 (0.39, 0.80)
9	31	20	11	0.65 (0.45, 0.81)

Supplementary Table S5. Results of nine lateral flow immunoassays (LFIA) devices, tested with plasma classified as positive and negative using ELISA as an alternative reference standard (n=81-90 per LFIA device). Different manufacturers are designated A-I. 95% confidence intervals (CI) are presented for each point estimate.

Assay	ELISA-positive		ELISA-negative		Sensitivity (95% CI)	Specificity (95% CI)
	True positive	False negative	True negative	False positive		
1	18	9	56	0	67 (46,83)	100 (94,100)
2	23	10	54	1	70 (51,84)	98 (90,>99)
3	21	6	54	2	78 (58,91)	96 (88,>99)
4	25	7	55	1	78 (60,91)	98 (90,>99)
5	19	6	54	2	76 (55,91)	96 (88,>99)
6	19	6	54	2	76 (55,81)	96 (88,>99)
7	23	4	53	3	85 (66,96)	95 (85,99)
8	17	9	55	1	65 (44,83)	98 (90,>99)
9	22	12	52	4	65 (46,80)	93 (83,98)

Supplementary table S6: Results of all assays performed and relevant metadata. Provided as separate supplementary_table_s6.xlsx file.

References

1. A Study Exploring Whooping Cough Protection in Children and Adults - ClinicalTrials.gov. [cited 2020 Apr 9]; https://clinicaltrials.gov/ct2/show/NCT03697798.

2. WHO. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf [accessed 10 Apr 2020]. 2020.