SUPPLEMENTARY SECTION

ELISA METHODOLOGIES

Spike protein ELISA

Antigen specific IgG antibodies were measured in all 314 sera using in-house standardised supersensitive IgG ELISA. In brief, 96 well binding plates (Greiner, Kremsmünster, Austria) were coated for 1 hour at 37°C, with antigen (1 µg/mL recombinant SARS-CoV-2 spike protein) for sample wells and with capture antibody (1 µg/mL anti-human kappa and lambda light chain specific mouse antibodies) (Southern Biotech, Birmingham, AL) for control wells, both diluted in 1x PBS.

Plates were washed with 1xPBS and blocked with goat assay buffer (5% Goat Serum, 1% Tween 20 in D-PBS) for 1 hour before addition of sera starting at 1:50 dilution to antigen coated wells and serial dilution of (1:10) of immunoglobulin standards (purified human IgG starting at 1µg/mL) were added in triplicate to kappa/lambda capture antibody-coated wells and incubated for 1 hour at 37°C.

Secondary antibody, biotin-conjugated anti-human IgG (Southern Biotech, Birmingham, AL), was added and incubated for 1 hour at 37°C before addition of Streptavidin-HRP (R&D Systems, Minneapolis, MN) at 1:200 for 1 hour at 37°C. Plates were developed with SureBlue TMB substrate (KPL, Insight Biotechnology, London, UK). The reaction was stopped after 5 minutes by adding TMB stop solution (KPL, Insight Biotechnology, London, UK), and the absorbance read at 450 nm on a VersaMax 96 well microplate reader (Molecular Devices, Sunnyvale, CA).

The OD 450 nm values obtained from known ng/ml concentrations of human IgG antibody standard in the control wells were used to generate a standard curve. Using the curve, the ODs from human serum samples binding to recombinant spike protein in sample wells were assigned ng/ml concentrations of spike-specific IgG.

The ELISA data were expressed as positive if the blank-subtracted OD 450 nm was above the pre-determined cut-off of OD 0·2 nm and values were on the linear range of the curve. The 0.2 OD cut-off was obtained from internal validation, in which hundreds of samples were run to generate standard curves to determine the cut-off. To ensure accuracy, a positive control (composed of positive pooled serum samples) and a negative serum control was run with each plate. Negative controls (coated with the antigen or kappa/lambda, but no samples added to them), were also run as an additional control. Specificity analysis on 105 pre-pandemic sera found a low level cross reactivity in 4% of samples, giving a specificity of 96%.

Analyses of the data were performed using SoftMax Pro GxP software (version 6.5, Molecular Devices, Sunnyvale, CA).

Double antigen bridging assay (hybrid DABA)

The Imperial Hybrid DABA is a sequential two step double antigen binding assay (DABA) for the detection and measurement of antibody directed to the receptor binding domain of SARS-CoV-2. It employs purified proteins expressed and gifted by the Crick Institute, London.

Solid phase 96 microwells plates (NUNC Immunomodule, U8 Maxisorp wells) were coated with the first of the antigens at a concentration of 5µg/ml (MicroImmune Coating Buffer; ClinTech, Guildford, UK) then washed, blocked (3-4 hours at 37°C in a moist box), aspirated and dried. Coating concentration was optimised for the S1 Crick coating through a checker board experiment with every production of conjugate and every batch of antigen. 50µl of sample diluent (MicroImmune Sample Diluent; ClinTech, Guildford, UK) and 50µl of control or test sera are together added to their respective wells. Plates are incubated for 1 hour at 37°C then washed (Washing buffer; ClinTech, Guildford,UK) followed by addition of an HRPO conjugate of the second protein appropriately diluted and incubated for 1hour at 37°C.

Plates are then washed five times and 100µl of TMB substrate (ClinTech, Guildford, UK) added, incubated for 30 minutes at 37°C, after which the reaction is stopped and absorbance measured at 450nm and 630nm.

The cut-off is established by adding 0.1 to the average of optical density (OD) obtained for the negative controls, assayed in triplicate in each run. The signal/cut-off value for each sample is determined by dividing the sample OD by the cut-off. A sample is considered reactive and to contain anti-RBD if S/CO ≥ 1.

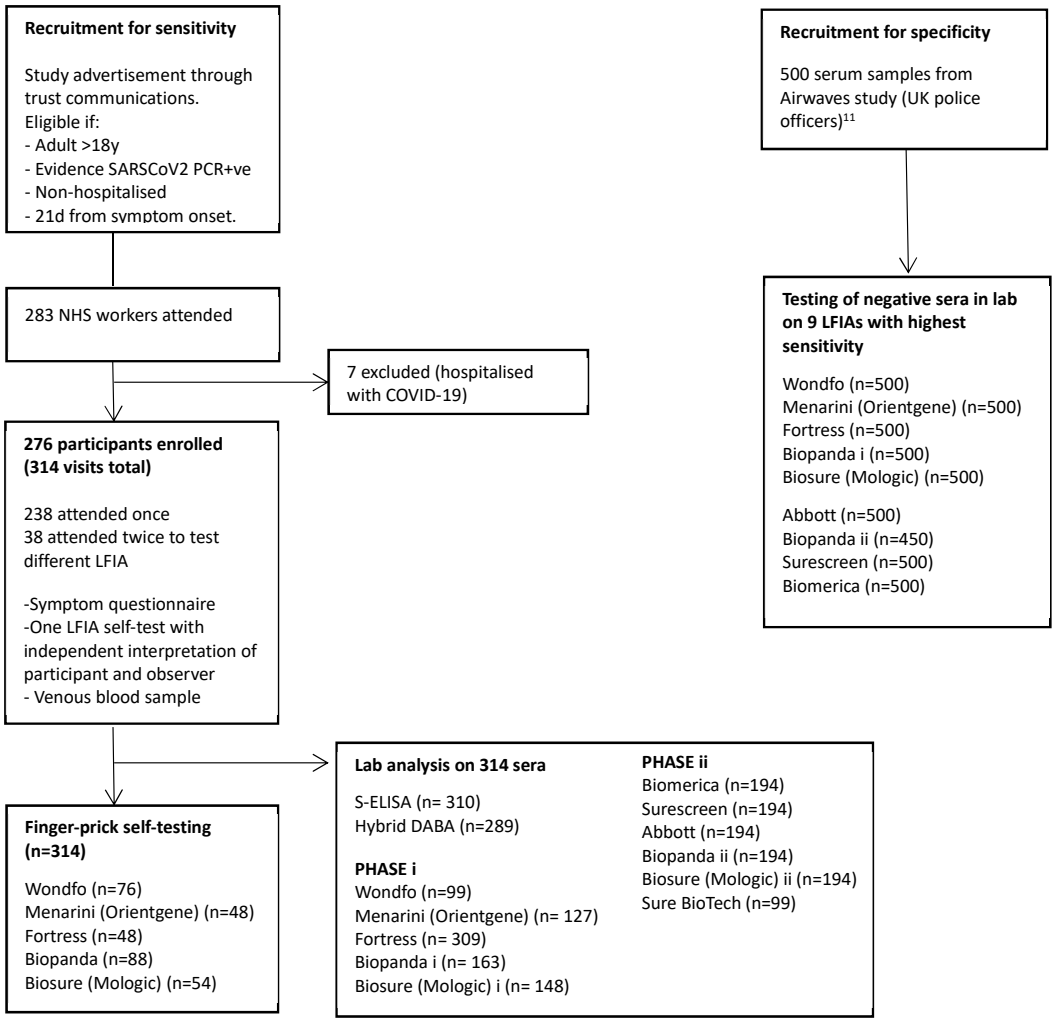
To evaluate assay specificity, the Hybrid DABA was tested on stored plasma and serum samples pre-dating the SARS-CoV-2 outbreak (n=825) comprising: 1) plasma samples from blood donors donated by NHSBT, Scotland (n=94); 2) serum samples from Airwaves study[13] (n=498); 3) plasma samples from antenatal screening (n=100); 4) plasma from HTLV-1 infected patients (n=133). None of these samples tested positive, giving a specificity of 100%.

***Supplementary table i:** lateral flow assay product specifications & reported performance*

LFIA	Product specifications	Manufacturer reported sensitivity (IgG; >21d from symptom onset)	Manufacturer reported specificity	Published performance
WONDFO Guangzhou Wondfo Biotech (Guangzhou, China) SARS-CoV-2 Antibody test (lateral flow method) Catalog no. W195 IgM/IgG combined Target antigen(s): Spike (S) protein)	Transfer 10µl whole blood, serum or plasma specimen to the small well, and then add 2-3 drops (80µl) of buffer solution to the large well. Read result no earlier than 15 minutes and no later than 20 minutes.	86.4% (82.51 - 89.58) (n=361)	99.57% (97.63 – 99.92) (n=235)	1. Sensitivity 77.1% (64/83) (Fingerprick; Hospitalised); specificity: 98.0% (2/100) (100 sera from 2012)[27] 2. Sensitivity: 81.8% (n=11) (Serum; Hospitalised) Specificity: 99.1 (n=106)[4] 3. Sensitivity: 86.4% (95% CI: 82.51-89.58%) (n=91) Specificity: 99.57% (95% CI: 97.63-99.92%) (n=92)[28]
MENARINI **(**Orientgene) Zhejiang Orient Gene Biotech Co Ltd REF GCCOV-402a Separate IgM and IgG Target: S1, S2 and Nucleocapsid (N) proteins	Add 5µl of serum/plasma or 10µl of whole blood to sample well. Then add 2 drops (about 80µl) of sample buffer to the buffer well immediately. Read result in 10 minutes, no later than 15m.	97.2% (85.4-99.9) (n=36)	100% (76.8-100) (n=14)	1. Sensitivity: 93.1 (27/29) (Sera; Hospitalised) Specificity: 98.75% (100 sera)[29] 2. Sensitivity: 91.6% (n=90) Specificity: 100% (n=9)[30]
FORTRESS Fortress Diagnostics COVID-19 TOTAL Ab Device Product Code: COVID010 / COVID020 Separate IgM and IgG Target antigen: S protein	Add 5-10µl of whole blood, serum or plasma into the specimen window. Immediately add two drops of diluent buffer into the buffer window. Read result 15m after specimen and buffer loading.	95.6% (90.7-98.4) (n=137)	95.2% (91.4-97.7) (n=209)	No published data
BIOPANDA COVID-19 Rapid Ab test Product Number: RAPG-COV-019 Separate IgM and IgG Target: S and N proteins	Add one drop of serum/plasma (10µl) or two drops of blood (20µl) (whole blood or fingerprick) or apply one drop of blood directly from finger to well of the cassette. Add two drops of buffer to the sample well. Read result after 10m	100% (90-100) (n=35)	98.3% (91.1 - 99.9) (n=60)	No published data
BIOSURE (Mologic)* COVID-19 Self-Test IgG only Target: N protein	Touch tip of test device onto drop of blood or serum until tip fills. Pierce the foil top of the buffer pot with the tip of the test device and push down hard (to bottom of buffer pot). Test requires 10m to run.	96.0% (79.65 – 99.90) (n=25)	98.8% (96.63-99.76) (n=257)	No published data

*Commercial Biosure kit comes in box with device holder and reading card. Clinic self-tests in this study were performed with the device alone.

***Supplementary figure i:** Flow of participants*



Supplementary table ii: symptom data

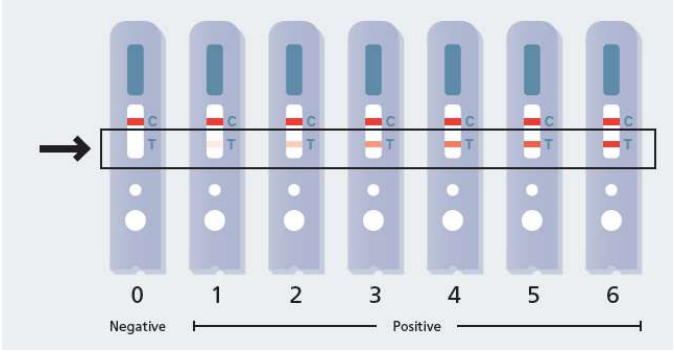
Symptoms, n(%)	
Decrease in appetite	146 (46)
Blocked nose	111 (35)
Loss of smell	206 (66)
Loss of taste	188 (60)
Sore throat	148 (47)
Headache	190 (61)
Shortness of breath	129 (41)
Persistent cough	144 (46)
Fever	192 (61)
Lethargy	245 (78)
Severe fatigue	120 (38)
Achy muscles	190 (61)
Nausea/vomiting	54 (17)
Diarrhoea	100 (32)
Abdominal pain	115 (37)
Runny nose	87 (28)
Sneezing	85 (27)
Sore eyes	58 (18)
Numbness or tingling	31 (10)
Hoarse voice	40 (13)
Dizziness	87 (28)
Tightness in chest	103 (33)
Chest pain	49 (16)
Heaviness in arms/legs	79 (25)
Chills	113 (36)
Difficulty sleeping	80 (25)
None of these	8 (3)

Supplementary table iii: inter-rater agreement in lab for test interpretation (7-point scale from 0 to 6, see suppl fig, 2)

	LFIA band reader i	LFIA band reader ii
001	0	0
002	0	0
004	1	2
005	2	3
006	1	1
007	4	4
008	1	2
009	0	0
010	3	3
011	1	1
012	3	3
013	3	3
014	5	5
015	3	3
016	1	1
017	1	1
018	3	3
019	1	1
020	2	2
021	0	0

Categorical score (0-6): Kappa = 0.81 (almost perfect agreement)
Binary score (0/1): Kappa = 1.0 (perfect agreement)

***Supplementary figure ii:** visual scoring system*

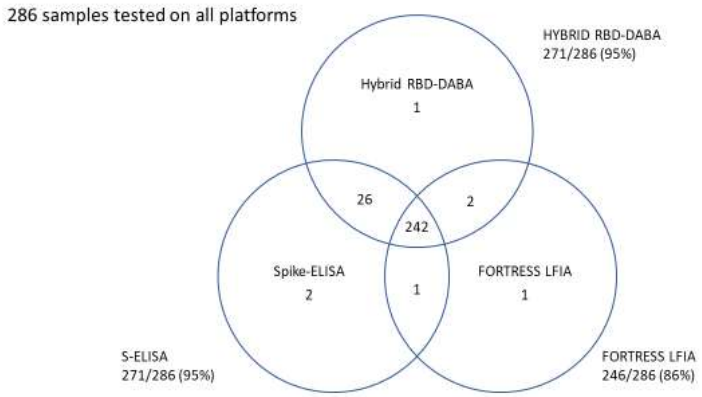


***Supplementary Table iv:** LFIA sensitivity*

	Test	Positive, n/N(%)	Sensitivity (%) against reference standards (95%CI)			
			PCR confirmed	S-ELISA	Hybrid DABA	S-ELISA and/or hybrid DABA
Wondfo SARS-CoV-2 Ab test (LFIA) IgG/M combined	Fingerprick, self-read	16/76 (21)	21.1 (12.5-31.9)	22.5 (13.5-34.0)	21.4 (12.5-32.9)	21.9 (13.1-33.1)
	Fingerprick, observer-read	16/76 (21)	21.1 (12.5-31.9)	22.5 (13.5-34.0)	21.4 (12.5-32.9)	21.9 (13.1-33.1)
	Lab serum	75/99 (76)	75.8 (66.1-83.8)	79.3 (69.6-87.1)	79.3 (69.3-87.3)	79.8 (70.2-87.4)
	Lab whole blood	45/84 (54)	53.6 (42.4-64.5)	57.1 (45.4-68.4)	60.6 (48.3-72.0)	57.1 (45.4-68.4)
Menarini Zhejiang Orient Gene (lateral flow) (IgG&M)	Fingerprick, self-read	44/48 (92)	91.7 (80.0-97.7)	95.6 (84.9-99.5)	97.7 (87.7-99.9)	95.6 (84.9-99.5)
	Fingerprick, observer-read	44/48 (92)	91.7 (80.0-97.7)	95.6 (84.9-99.5)	97.7 (87.7-99.9)	95.6 (84.9-99.5)
	Lab serum	113/127 (89)	89.0 (82.2-93.8)	93.3 (87.2-97.1)	94.7 (88.8-98.0)	92.6 (86.3-96.5)
	Lab whole blood	66/72 (92)	91.7 (82.7-96.9)	97.0 (89.6-99.6)	100 (94.4-100)	97.0 (89.6-99.6)
Fortress Diagnostics COVID-19 total Ab (IgG&M)	Fingerprick, self-read	38/48 (79)	79.2 (65.0-89.5)	84.4 (70.5-93.5)	88.4 (74.9-96.1)	84.4 (70.5-93.5)
	Fingerprick, observer-read	37/48 (77)	77.1 (62.7-88.0)	82.2 (67.9-92.0)	86.0 (72.1-94.7)	82.2 (67.9-92.0)
	Lab serum	258/307 (84)	84.0 (79.5-88.0)	88.2 (83.9-91.7)	89.3 (84.9-92.7)	87.6 (83.3-91.2)
	Lab whole blood					
Biopanda COVID-19 Rapid antibody test (IgG&M)	Fingerprick, self-read	57/88 (65)	64.8 (53.9-74.7)	66.7 (55.5-76.6)	68.6 (56.4-79.1)	66.7 (55.5-76.6)
	Fingerprick, observer-read	65/88 (74)	73.9 (63.4-82.7)	76.2 (65.7-84.8)	77.1 (65.6-86.3)	76.2 (65.7-84.8)
	Lab serum	102/163 (63)	62.6 (54.7-70.0)	64.9 (56.8-72.4)	65.5 (56.9-73.3)	64.7 (56.7-72.2)
	Lab whole blood	29/44 (66)	65.9 (50.1-79.5)	70.0 (53.5-83.4)	72.2 (54.8-85.8)	70.7 (54.5-83.9)
Biosure (Mologic) COVID-19 antibody self-test (IgG only)	Fingerprick, self-read	32/54 (59)	59.3 (45.0-72.4)	60.4 (45.3-74.2)	60.4 (45.3-74.2)	61.2 (46.2-74.8)
	Fingerprick, observer-read	40/54 (74)	74.1 (60.3-85.0)	79.2 (65.0-89.5)	79.2 (65.0-89.5)	79.6 (65.7-89.8)
	Lab serum	99/148 (67)	66.9 (58.7-74.4)	71.3 (62.9-78.7)	70.5 (61.9-78.1)	70.5 (62.2-77.9)
	Lab whole blood	35/50 (70)	70.0 (55.4-82.1)	72.3 (57.4-84.4)	72.1 (56.3-84.7)	70.8 (55.9-83.0)

95%CI, 95% Binomial exact confidence interval

Supplementary figure iii: Venn diagram illustrating positivity agreement for lab platforms and Fortress LFIA

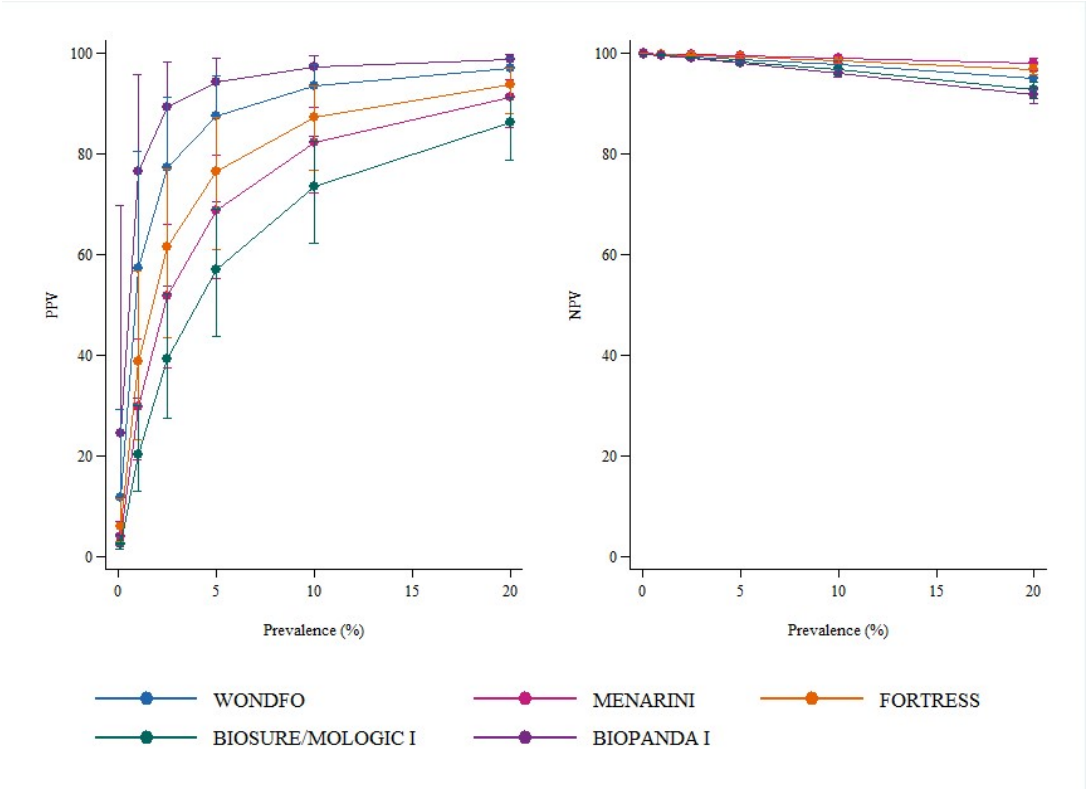


Supplementary table v: 2x2 contingency tables for paired lab and clinic results vs i) PCR-confirmed & ii) S-ELISA and/or hybrid DABA

	PCR-confirmed			S-ELISA or hybrid DABA		
Wondfo		Lab			Lab	
	Clinic	-	+	Clinic	-	+
	-	19	41	-	16	41
	+	1	15	+	1	15
Menarini		Lab			Lab	
	Clinic	-	+	Clinic	-	+
	-	2	2	-	1	1
	+	1	42	+	1	42
Fortress		Lab			Lab	
	Clinic	-	+	Clinic	-	+
	-	5	5	-	2	5
	+	1	37	+	1	37
Biopanda		Lab			Lab	
	Clinic	-	+	Clinic	-	+
	-	11	13	-	9	12
	+	6	38	+	6	38
Biosure		Lab			Lab	
	Clinic	-	+	Clinic	-	+
	-	8	11	-	6	10
	+	1	24	+	0	24

2x2 contingency tables for individuals with matched lab (serum) and clinic (fingerprick) LFIA test results

***Supplementary figure iv:** Positive and negative predictive values for different population seroprevalence.*



***Supplementary figure v:** antibody levels by S-ELISA and hybrid DABA against weeks since symptom onset*