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Combined point of care nucleic acid and antibody testing for SARS-CoV-2: a prospective cohort study in suspected moderate to severe COVID-19 disease.

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Abstract

Background

Rapid COVID-19 diagnosis in hospital is essential for patient management and identification of infectious patients to limit the potential for nosocomial transmission. The diagnosis is complicated by 30-50% of COVID-19 hospital admissions with negative nose/throat swabs for SARS-CoV-2 nucleic acid, frequently after the first week of illness when SARS-CoV-2 antibody responses become detectable. We assessed the diagnostic accuracy of combined rapid antibody point of care (POC) and nucleic acid assays for suspected COVID-19 disease in the emergency department.

Methods

We developed (i) an in vitro neutralization assay using a lentivirus expressing a genome encoding luciferase and pseudotyped with spike protein and (ii) an ELISA test to detect IgG antibodies to nucleocapsid (N) and spike (S) proteins from SARS-CoV-2. We tested two promising candidate lateral flow rapid fingerprick test with bands for IgG and IgM. We then prospectively recruited participants with suspected moderate to severe COVID-19 and tested for SARS-CoV-2 nucleic acid in a combined nasal/throat swab using the standard laboratory RT-PCR and a validated rapid nucleic acid test. Additionally, serum collected at admission was retrospectively tested by in vitro neutralization, ELISA and the candidate POC antibody tests. We determined the sensitivity and specificity of the individual and combined rapid POC diagnostic tests against a composite 'gold' standard of neutralisation and the standard laboratory RT-PCR.

Results

45 participants had specimens tested for nucleic acid in nose/throat swabs as well as stored sera for antibodies. Serum neutralisation assay, SARS-CoV-2 Spike IgG ELISA and the POC antibody test results were concordant. Using the composite gold standard, prevalence of COVID-19 disease was 53.3% (24/45). Median age was 73.5 (IQR 54.0-86.5) years in those with COVID-19 disease by our gold standard and 63.0 (IQR 41.0-72.0) years in those without disease. Median duration of symptoms was 7 days (IQR 1-8) in those with infection. The overall sensitivity of rapid NAAT diagnosis was 79.2% (95CI 57.8-92.9%). Sensitivity and specificity of the combined rapid POC diagnostic tests reached 100% (95CI 85.8-100) and 94.7% (95CI 74.0-99.0) overall.

Conclusions

Dual point of care SARS-CoV-2 testing can significantly improve diagnostic sensitivity, whilst maintaining high specificity. Rapid combined tests have the potential to transform our

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management of COVID-19, including inflammatory manifestations where nucleic acid test results are negative. A rapid combined approach will also aid recruitment into clinical trials and in prescribing therapeutics, particularly where potentially harmful immune modulators (including steroids) are used.

Introduction

As of the 5th of June 2020, 6.7 million people have been infected with SARS-CoV-2 with over 390 000 deaths[1]. The unprecedented numbers requiring SARS-CoV-2 testing has strained healthcare systems globally. There is currently no gold standard for diagnosis of COVID-19. Detection of SARS-CoV-2 by nucleic acid amplification testing (NAAT), is largely done by real time RT-PCR on nose/throat swabs in centralised laboratories. RT-PCR specimens need to be handled in biosafety level 3 category laboratory (BSL3) and then batch analysed. Given these bottlenecks, the turnaround time for this test is in the order of 2- 4 days[2]. NAAT tests from a single nose/throat swab are negative in up to 50% in patients who have CT changes consistent with COVID-19 and/or positive antibodies to SARS-CoV-2 [3-5]. The lack of detectable virus in upper airway samples is not only a serious barrier to making timely and safe decisions in the ER, but also leads to multiple swab samples being sent, frequently from the same anatomical site, leading to strain on virology laboratories. Additionally, recruitment into clinical trials for COVID-19 treatments has moved towards 'clinical diagnosis' for eligibility.

Multiple factors contribute to negative results by NAAT, including sampling technique and timing of the sampling in the disease course. The viral load in the upper respiratory tract frequently wanes by this point[6] and as seen in a case series from France, was undetectable in nose and throat swabs from 9 days of illness in 4 out of 5 patients[7]. Similarly, a case series from Germany found the detection rate by RT-PCR was <50% after 5 days since onset of illness[8]. A proportion of patients develop a secondary deterioration in clinical condition requiring hospitalisation and respiratory support, at a time when immune pathology is thought to be dominate rather than direct pathology related to viral replication [7, 9].

The antibody response to SARS-CoV-2 is detectable 6 days from infection[10]. Antibody based diagnosis of COVID-19 shows increasing sensitivity in the latter part of the disease course when NAAT testing on nose/throat samples is more likely to be negative[11-14]. One study reported that combined lab based RT-PCR with lab based antibody testing could

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increase sensitivity for COVID-19 diagnosis from 67.1% to 99.4% in hospitalised patients[15]. However, lab antibody testing also has a turnaround time of a day or more, and rapid diagnosis and triage of patients requiring hospitalisation is needed in order to avoid overwhelming the diagnostic and isolation capacities of hospitals, especially during periods when influenza is co-circulating.

We previously evaluated the diagnostic accuracy of the SAMBA II SARS-CoV-2 rapid test compared with the standard laboratory RT-PCR and found similar accuracy and a turnaround time of 2-3 hours even in real world settings [2]. Several studies have now performed head-to-head comparisons of immuno-chromatographic lateral flow immunoassays (LFIAs)[12-14, 16]. These assays are cheap to manufacture and give a binary positive/negative result, thereby lending themselves well to point of care (POC) testing. However, they have variable performance and in general they are negative in the early phase of illness, but highly sensitive in the later stage of illness[12-14, 16]. In this study we evaluated the diagnostic performance of a POC combination comprising NAAT and LFA antibody testing against a composite gold standard of laboratory RT-PCR and a serum neutralisation assay.

Results

45 prospectively recruited participants with suspected moderate to severe COVID-19 disease had specimens tested for nucleic acid in nose/throat swabs as well as stored sera for antibodies. Samples at hospital admission were collected at a median of 7 (IQR 7-13) days after illness onset. The sera from 42.2% (19/45) participants showed strong neutralising antibody response against SARS-CoV-2 spike protein pseudotyped virus infection in a neutralization assay (Figure 1A). 26 participants' sera showed no neutralising response (Figure 1B). The neutralisation ability of participants' sera was compared with an ELISA IgG assay (Supplementary Figure 1) detecting Spike antibodies. Figure 1C confirms significant association between positive results in both assays ($p<0.0001$). Importantly, Figures 1D and E show concordance between the point of care antibody test result and both ELISA ($p<0.0001$) and neutralisation assays, $p<0.0025$ (Figure 1D, E). The neutralisation assay did not cross react with SARS-CoV-1 (Supplementary Figure 2).

53.3% (24/45) of participants had COVID-19 disease, as determined by the composite gold standard. Median age was 73.5 (IQR 54.0-86.5) years in those with SARS-CoV-2 infection by our gold standard and 63.0 (IQR 41.0-72.0) years in those without disease (Table1). CRP

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and procalcitonin were significantly higher in confirmed COVID-19 patients and 'classical' chest radiograph appearances were more common in confirmed COVID-19 patients (Table1, p<0.001). However, 6/24 (25%) had normal or indeterminate chest radiographs in the confirmed COVID-19 group.

The overall positivity rate of the rapid nucleic acid test was 79.2% (95% CI 57.8-92.9), decreasing from 88.9% (95% CI 65.3-98.6) in days 1-7 of illness to 50.0% (95% CI 11.8-88.2) in days 8-28 of illness (Table 2). When the COVIDIX IgG/IgM rapid test was combined with NAAT, the positivity rate increased to 100% (95% CI 81.5-100) in days 1-7 of illness and 100% (95% CI 54.1-100) in days 8-28 of illness. Specificity was 90.0% (95% CI 55.5-99.7) in the first 7 days of illness and 81.8% (95% CI 48.2-97.7) at 8-28 days of illness when the combined POC test results were considered. Overall specificity of the combined rapid tests was 85.7% (95%CI 63.7-97.0). On closer analysis of 'false positive' results for the COVIDIX SARS-CoV-2 IgG/IgM test, we noted that 2/3 individuals had normal chest radiographs and the third had a pulmonary embolus diagnosed on CT pulmonary angiography. All had normal lymphocyte counts (Supplementary table 1).

Three participants had stored samples available for testing at multiple time points in their illness (Figure 2). Two individuals were sampled from early after symptom onset and the third presented three weeks into illness. In the first two (Figure 2A-F), we observed an increase in neutralisation activity over time that was mirrored by band intensities on the rapid POC antibody test. As expected IgM bands arose early on with IgG following closely. In the individual presenting 21 days into illness (Figure 2G-I), only IgG was detected with the rapid POC antibody test and as expected band intensity did not increase with time.

Given the need for multiple options under current demand for such tests, we next decided to use an alternative rapid LFA antibody test in combination with the SAMBA II NAAT. SureScreen SARS-CoV-2 IgG/IgM test (Derby, UK) was recently validated with ELISA IgG and demonstrated a very good sensitivity and specificity profile compared to five other tests on stored sera from acute infection[17]. We compared ELISA IgG and serum neutralisation titres (EC50) against this POC for 43 of the original serum samples (Supplementary Figure 3). Use of SureScreen SARS-CoV-2 IgG/IgM test in combination with SAMBA II NAAT resulted in similar sensitivity (100% [95% CI 85.8-100%]) but higher specificity (94.7% [95% CI 74.0-99.0%]) as compared to the COVIDIX SARS-CoV-2 IgG/IgM test (Table 2).

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Discussion

Here we have shown that NAAT testing with antibody detection can improve diagnosis of COVID-19 in moderate to severe suspected cases, but more importantly that accurate diagnosis can be achieved with combined rapid tests. Overall positivity in nose/throat swab samples was around 80% with NAAT testing alone and 100% with a combined approach of rapid NAAT testing and either of two fingerprick blood/serum rapid antibody tests. Specificity of the combined approach was 85-95% overall. As expected, nucleic acid detection in nose/throat samples was highest in the first few days (100% for SAMBA II SARS-CoV-2 test in the first 3 days after symptom onset). Conversely antibody detection by LFA increased over time.

A strength of this study is the use of serum neutralisation, a phenotypic test for functionality of antibodies, as part of a composite gold standard for defining COVID-19 disease. This assay was carefully validated against a recently described ELISA method for SARS-CoV-2 IgG detection that is now used globally[18]. We also demonstrated that sera from participants did not neutralise SARS-CoV-1.

Use of antibody tests for COVID-19 diagnosis in hospitals have been limited for a number of reasons. Firstly, we know from SARS-CoV-1 that previous humoral immunity to HCoV OC43 and 229E can elicit a cross reactive antibody response to N of SARS-CoV-1 in up to 14% of people tested in cross-sectional studies[19], and previous exposure to HCoV can rarely elicit an antibody response cross reaction to the N and S proteins of SARS- CoV-2 [17, 20]. Secondly, antibody tests do not achieve the same detection rates as nucleic acid based tests early in infection, as humoral responses take time to develop following viral antigenic stimulation. However, later in disease IgG reaches 100% sensitivity by day 6[10] and this is useful in cases with immune mediated inflammatory disease where RT-PCR on respiratory samples is often negative, for example in the recently described Kawasaki-like syndrome named PIMS (paediatric inflammatory multi-system syndrome) [21].

CT scanning has previously been shown to be highly sensitive[5], though few countries have the resources for large scale CT based screening. In our study chest radiographs were statistically more likely to show changes associated with COVID-19, but a quarter of chest radiographs in the confirmed COVID-19 group were normal or indeterminate.

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This study had limited numbers of participants, though patients were distributed well by symptom onset and part of a clinical trial with complete data. We tested stored sera rather than whole finger prick blood, though this was intentional given the caution needed in interpreting antibody tests and potential cross reactivity of antibodies. Although SARS-CoV2 ELISA testing of our pre 2020 sera did reveal occasional N and S reactivity to SARS- CoV-2 (supplementary table 2), these samples were negative on the rapid antibody testing. In light of our data, prospective evaluation on a finger prick sample is now warranted on a larger scale in patients with moderate to severe disease. At present we cannot speculate on the diagnostic accuracy of the antibody or NAAT tests in mild disease.

We envisage a deployment approach whereby both test samples, finger prick blood and nose/throat swab, are taken at the same time on admission to hospital. The finger prick antibody test result is available within 15 minutes and is highly specific; therefore in an individual with classical features and a positive antibody test result can be acted upon confidently, for example movement to a COVID-19 area, or recruitment into a clinical treatment study. The NAAT result following shortly after will assist in diagnosis for early infections where antibody testing is negative. NAAT is also expected to be more valuable than antibody tests in milder cases given severity appears to correlate with magnitude of antibody responses [17, 22].

A combined rapid testing approach may have significant benefits in low resource settings where centralised virology laboratories are scarce and the epidemic is expanding. In addition, it removes the need for repeated nose/throat swabbing which may generate aerosols and lead to transmission. We envisage the combined rapid testing approach being important for safe and quick patient recruitment to clinical trials for COVID-19, specifically where potentially harmful treatments such as immune modulators are being tested. Rapid combined tests could be transformative in diagnosis and management of moderate to severe COVID-19 disease requiring hospitalisation, particularly as diverse manifestations of disease emerge.

Methods

Cell lines

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293T cells were cultured in DMEM complete (DMEM supplemented with 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 10% FCS).

Pseudotype virus preparation

Viral vectors were prepared by transfection of 293T cells by using Fugene HD transfection reagent (Promega) as follows. Confluent 293T cells were transfected with a mixture of 11ul of Fugene HD, 1ug of pCAGGS_SARS-CoV-2_Spike, 1ug of p8.91 HIV-1 gag-pol expression vector[23, 24], and 1.5ug of pCSFLW (expressing the firefly luciferase reporter gene with the HIV-1 packaging signal). Viral supernatant was collected at 48 and 72h after transfection, filtered through 0.45um filter and stored at -80˚C. The 50% tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2 pseudovirus was determined using using Steady-Glo Luciferase assay system (Promega).

Pseudotype neutralisation assay

Spike pseudotype assays have been shown to have similar characteristics as neutralization testing using fully infectious wild type SARS-CoV-2[25].Virus neutralization assays were performed on 293T cell transiently transfected with ACE2 and TMPRSS2 using SARS-CoV-2 Spike pseudotyped virus expressing luciferase. Pseudovirus was incubated with serial dilution of heat inactivated human serum samples from COVID-19 suspected individuals in duplicates for 1h at 37˚C. Virus and cell only controls were also included. Then, freshly trypsinized 293T ACE2/TMPRSS2 expressing cells were added to each well. Following 48h incubation in a 5% CO_2 environment at 37°C, the luminescence was measured using Steady-Glo Luciferase assay system (Promega). The 50% inhibitory dilution (EC_{50}) was defined as the serum dilution at which the relative light units (RLUs) were reduced by 50% compared with the virus control wells (virus + cells) after subtraction of the background RLUs in the control groups with cells only. The EC_{50} values were calculated with non-linear regression, log (inhibitor) vs. normalized response using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA). The neutralisation assay was positive if the serum achieved at least 50% inhibition at 1 in 3 dilution of the SARS-CoV-2 spike protein pseudotyped virus in the neutralisation assay. The neutralisation result was negative if it failed to achieve 50% inhibition at 1 in 3 dilution.

Enzyme-linked immunosorbent assay (ELISA)

We developed an ELISA targeting the SARS-CoV-2 Spike and N proteins. Trimeric spike protein antigen used in ELISA assays consists of the complete S protein ectodomain with a C-terminal extension containing a TEV protease cleavage site, a T4 trimerization foldon and

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a hexa-histidine tag. The S1/S2 cleavage site with amino acid sequence PRRAR was replaced with a single Arginine residue and stabilizing Proline mutants were inserted at positions 986 and 987. Spike protein was expressed and purified from Expi293 cells (Thermo Fisher). N protein consisting of residues 45-365 was initially expressed as a His-TEV-SUMO-fusion. After Ni-NTA purification, the tag was removed by TEV proteolysis and the cleaved tagless protein further purified on Heparin and gel filtration columns.

The ELISAs were in a stepwise process; a positivity screen was followed by endpoint titre as previously described[18]. Briefly, 96-well EIA/RIA plates (Corning, Sigma) were coated with PBS or 0.1µg per well of antigen at 4°C overnight. Coating solution was removed, and wells were blocked with 3% skimmed milk prepared in PBS with 0.1% Tween 20 (PBST) at ambient temperature for 1 hour. Previously inactivated serum samples (56°C for 1 hour) were diluted to 1:60 or serially diluted by 3-fold, six times in 1% skimmed milk in PBST. Blocking solution was aspirated and the diluted sera was added to the plates and incubated for 2 hours at ambient temperature. Diluted sera were removed, and plates were washed three times with PBST. Goat anti-human IgG secondary antibody-Peroxidase (Fc-specific, Sigma) prepared at 1:3,000 in PBST was added and plates were incubated for 1 hour at ambient temperature. Plates were washed three times with PBST. ELISAs were developed using 3,5,3′,5′- tetramethylbenzidine (TMB, ThermoScientific); reactions were stopped after 10 minutes using 0.16M Sulfuric acid. The optical density at 450 nm (OD450) was measured using a Spectramax i3 plate reader. The absorbance values for each sample were determined by subtracting OD values from uncoated wells. All data analyses were performed using Prism 8 version 8.4.2 (GraphPad).

COVIDIX 2019 SARS-CoV-2 IgG/IgM Test (COVIDIX Healthcare, Cambridge, UK).

This colloidal-gold lateral flow immunoassay is designed to detect IgG and IgM to SARS-CoV-2. It was used according to the manufacturer's instructions. 10µl of serum was added to the test well followed by 2 drops of the manufacturer's proprietary buffer. Results were read as the presence or absence of a colored band the results window as IgM positive-control and IgM test bands present, as IgG positive-control and IgG test bands present or negative-control band only.

In order to rule out cross reactivity of this test with seasonal coronavirus antibodies we tested 19 stored specimens from before 2020, some of which had N and S protein SARS-CoV-2 cross reactivity (Supplementary table 2). For quantification of IgG and IgM band density in COVIDIX 2019 nCoV IgG/IgM Test, high resolution images of completed POC antibody test cassettes were acquired using ChemiDoc MP Imaging System (Bio-Rad) at 20min post-

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addition of the human serum. Band intensities were analysed using Image Lab software (Bio-Rad).

SureScreen SARS-CoV-2 IgG/IgM Test (SureScreen Diagnostics Ltd, Derby, UK). This colloidal-gold lateral flow immunoassay is designed to detect IgG and IgM to SARS-CoV-2. It was used according to the manufacturer's instructions. 10µl of serum was added to the test well followed by 2 drops of the manufacturer's proprietary buffer. Results were read as the presence or absence of a colored band the results window as IgM positive-control and IgM test bands present, as IgG positive-control and IgG test bands present or negative-control band only. It has been previously validated against historical controls and in serum from confirmed PCR positive COVID-19 cases[17].

Participants

The study participants were part of the COVIDx trial[2], a prospective analytical study which compared SAMBA II SARS-CoV-2 point of care testing compared to the standard lab RT-PCR test for the detection of SARS-CoV-2 in participants admitted to Cambridge University Hospitals NHS Foundation Trust (CUH) with a possible diagnosis of COVID-19. The COVIDx study included consecutive participants were recruited during 12-hour day shifts over a duration of 4 weeks from the 6th of April 2020 to the 2nd of May 2020. We recruited adults (>16 years old) presenting to the emergency department or acute medical assessment unit as a possible case of COVID-19 infection. This included participants who met the Public Heath England (PHE) definition of a possible COVID-19 case (see supplemental methods). This was later expanded to include any adult requiring hospital admission and who was symptomatic of SARS-CoV-2 infection, demonstrated by clinical or radiological findings. [2]. 48 participants who had available stored sera were included in this sub-study and underwent further antibody testing. The laboratory standard RT- PCR test, developed by public health England (PHE), targeting the RdRp gene was performed on a combined nose/throat swab on parallel. This test has an estimated limit of detection of 320 copies/ml. SAMBA II SARS-CoV-2 testing was performed on a combined nose/throat swab collected by dry sterile swab and inactivated in a proprietary buffer at point of sampling. SAMBA II SARS-CoV-2 targets 2 genes- Orf1 and the N genes and uses nucleic acid sequence based amplification to detect SARS-CoV-2 RNA, with limit of detection of 250 copies/ml.

Analyses

The sensitivity and specificity of SAMBA II SARS-CoV-2 test and COVIDIX SARS-CoV-2 IgG/IgM Test or SureScreen SARS-CoV-2 IgG/IgM Test for diagnosing COVID-19 were

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calculated alone and then in combination along with binomial 95% confidence intervals (CI). A composite gold standard was used - standard lab RT-PCR and a neutralisation assay. Descriptive analyses of clinical and demographic data are presented as median and interquartile range (IQR) when continuous and as frequency and proportion (%) when categorical. The differences in continuous and categorical data were tested using Wilcoxon rank sum and Chi-square test respectively. The correlation between ELISA and neutralisation assay was determined using the Pearson Correlation coefficient. Statistical analysis were conducted using Stata (version 13), with additional plots generated using GraphPad Prism.

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Table 1: Characteristics of participants in prospective study. COVID-19 status is based on composite gold standard test of nose/throat swab SARS-CoV-2 RT-PCR + Serum Neutralisation of pseudovirus bearing SARS-CoV-2 Spike. [§] Wilcoxon rank sum test used except where indicated. [a] Chi-square test.

	COVID-19 N=24	No COVID-19 N=21	P value [§]
Male sex (%)	14 (58.3)	9 (42.9)	0.30[a]
Median age (IQR) yrs	73.5 (54.0-86.5)	63.0 (41.0-72.0)	0.03
Median SpO2 (IQR) %	95.0 (92.5-96.0)	96.0 (94.0-98.0)	0.09
Median FiO2 (IQR)	0.21 (0.21-0.24)	0.21 (0.21-0.21)	0.40
Median PaO2 (IQR) Kpa	5.0 (3.0-9.1)	7.2 (3.8-9.0)	0.30
Median PaO2:FiO2 ratio (IQR)	20.5 (13.3-32.9)	30.9 (18.1-36.2)	0.09
Median Respiratory rate (IQR) breaths/min	22.0 (19.0-27.5)	20.0 (17.0-23.0)	0.06
Median heart rate (IQR) beats/min	86.0 (77.5-99.5)	88.0 (78.0- 107.0)	0.44
Median Systolic BP (IQR) mmHg	139.5 (117.5-149.0)	135.0 (119.0-152.0)	0.90
Median duration of illness (IQR) days	7 (1-8)	10 (3-14)	0.10
Median Hb (IQR) g/dL	12.9 (12.0-13.8)	13.1 (11.6-14.1)	0.46
Median WCC (IQR) $x10^9$/L	7.0 (5.0-8.0)	9.0 (7.0-14.0)	0.08
Median lymphocyte count (IQR) $x10^9$/L	0.8 (0.5-1.2)	1.2 (0.8-1.5)	0.12
Median platelet count (IQR) $x10^9$/L	213.5 (188.5-303.5)	271.0 (186.0-305.0)	0.59
Median Ferritin (IQR) µg/L	684.7 (206.2-1059.1)	112.3 (49.6-323.6)	0.02
Median Dimer (IQR) ng/mL	369.0 (254.0-974.0)	267.5 (66.0-550.5)	0.10
Median CRP (IQR) mg/L	72.0 (28.5-214.5)	12 (4.0-53.0)	0.004
Median procalcitonin (IQR) ng/mL	0.2 (0.1-0.6)	0.0 (0.0-0.1)	0.03
Radiological findings			
Normal	2 (8.3)	9 (42.9)	<0.001 [a]
Indeterminate	4 (16.7)	3 (14.3)	
Classic	18 (75.0)	3 (14.3)	
Non-COVID	0 (0.0)	6 (28.5)	

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Table 2: Individual and combined diagnostic accuracy of point of care rapid NAAT-based and antibody tests in participants presenting 1-7 days and 8-28 days after illness onset. Composite gold standard is laboratory RT-PCR and serum neutralisation. Sensitivity, specificity, PPV- positive predictive value, NPV-negative predictive values are presented with binomial exact 95% confidence intervals (CI). Highlighted in bold is

% (95% CI)	1-7 days N=28	8-28 days N=17	Overall N=45
SAMBA II SARS-CoV-2			
Sensitivity	88.9 (65.3-98.6)	50.0 (11.8-88.2)	79.2 (57.8-92.9)
Specify	100 (69.2-100)	100 (71.5-100)	100 (83.9-100)
PPV	100 (79.4-100)	100 (29.2-100)	100 (83.2-100)
NPV	83.3 (51.6-97.9)	78.6 (49.2-95.3)	80.0 (60.6-93.4)
COVIDIX Ig M & IgG			
Sensitivity	94.4 (72.7-99.9)	100 (54.1-100)	95.8 (78.9-99.9)
Specificity	90.0 (55.5-99.7)	81.8 (48.2-97.7)	85.7 (63.7-97.0)
PPV	94.4 (72.7-99.9)	75.0 (34.9-96.8)	88.5 (69.8-97.6)
NPV	90.0 (55.5-99.7)	100 (66.4-100)	94.7 (74.0-99.9)
SAMBA II SARS-CoV-2 + COVIDIX IgM &IgG			
Sensitivity	100 (81.5-100)	100 (54.1-100)	100 (85.8-100)
Specificity	90.0 (55.5-99.7)	81.8 (48.2-97.7)	85.7 (63.7-97.0)
PPV	94.7 (74.0-99.9)	75.0 (34.9-96.8)	88.9 (70.8-97.6)
NPV	100 (66.4-100)	100 (66.4-100)	100 (81.5-100)
SAMBA II SARS-CoV-2 & SureScreen IgM &IgG			
Sensitivity	**100 (81.5-100)**	**100 (54.1-100)**	**100 (85.8-100)**
Specificity	**88.9 (51.8-99.7)**	**100 (69.2-100)**	**94.7 (74.0-99.9)**
PPV	94.7 (74.0-99.9)	100 (54.1-100)	96.0 (79.6-99.9)
NPV	100.0 (63.1-100)	100 (69.2-100)	100 (81.5-100)

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Supplementary Table 1: clinical details of participants with false positive combined rapid testing (due to false positive rapid IgM IgG result).

Clinical features	#20	#35	#40
Radiology	Normal	Normal	Pulmonary embolus
Oxygen saturations (%)	95	88	97
PaO2/FiO2	42.9	41.9	32.4
Temperature (^{o}C)	37.0	37.9	36.9
Respiratory rate (breaths/min)	17	24	18
Lymphocyte count (x10^9 /L)	3.3	1.7	1.2
C-reactive protein (mg/L)	4	35	135

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Supplementary Table 2: ELISA optical density values for full length Spike (FL), Spike receptor binding domain (RBD), nucleocapsid (N). Positive (from confirmed positive) and negative (pooled human sera from pre 2020) control values are given.

Sample no	FL	RBD	N
1	0.95735	0.20455	0.5343
2	0.1217	0.1008	0.0746
3	0.2680	0.1300	0.1285
4	0.2511	0.0837	0.07445
5	0.10625	0.06625	0.4722
6	0.1561	0.08655	0.0927
7	1.12375	0.05785	0.40535
8	0.1432	0.0888	0.5842
9	0.49075	0.06505	0.32445
10	0.16075	0.03625	0.13485
11	0.08205	0.0504	0.07485
12	0.1956	0.23025	0.1748
13	0.1482	0.07115	0.05645
14	0.16075	0.078	1.00845
15	0.18015	0.09845	0.7598
16	0.26335	0.0693	0.38865
17	0.1864	0.18905	0.35065
18	0.1265	0.3684	0.18025
19	0.08425	0.06555	0.1378
positive	0.297	0.054	0.387
negative	2.704	2.150	2.337

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Ethical approval: COVIDx (NCT04326387) was approved by the East of England - Essex Research Ethics Committee (REC ref: 20/EE/0109**)**. Serum samples were obtained from patients attending Addenbrooke's Hospital with a suspected or confirmed diagnosis of COVID19. Ethical approval was obtained from the East of England – Cambridge Central Research Ethics Committee (REC ref 17/EE/0025).

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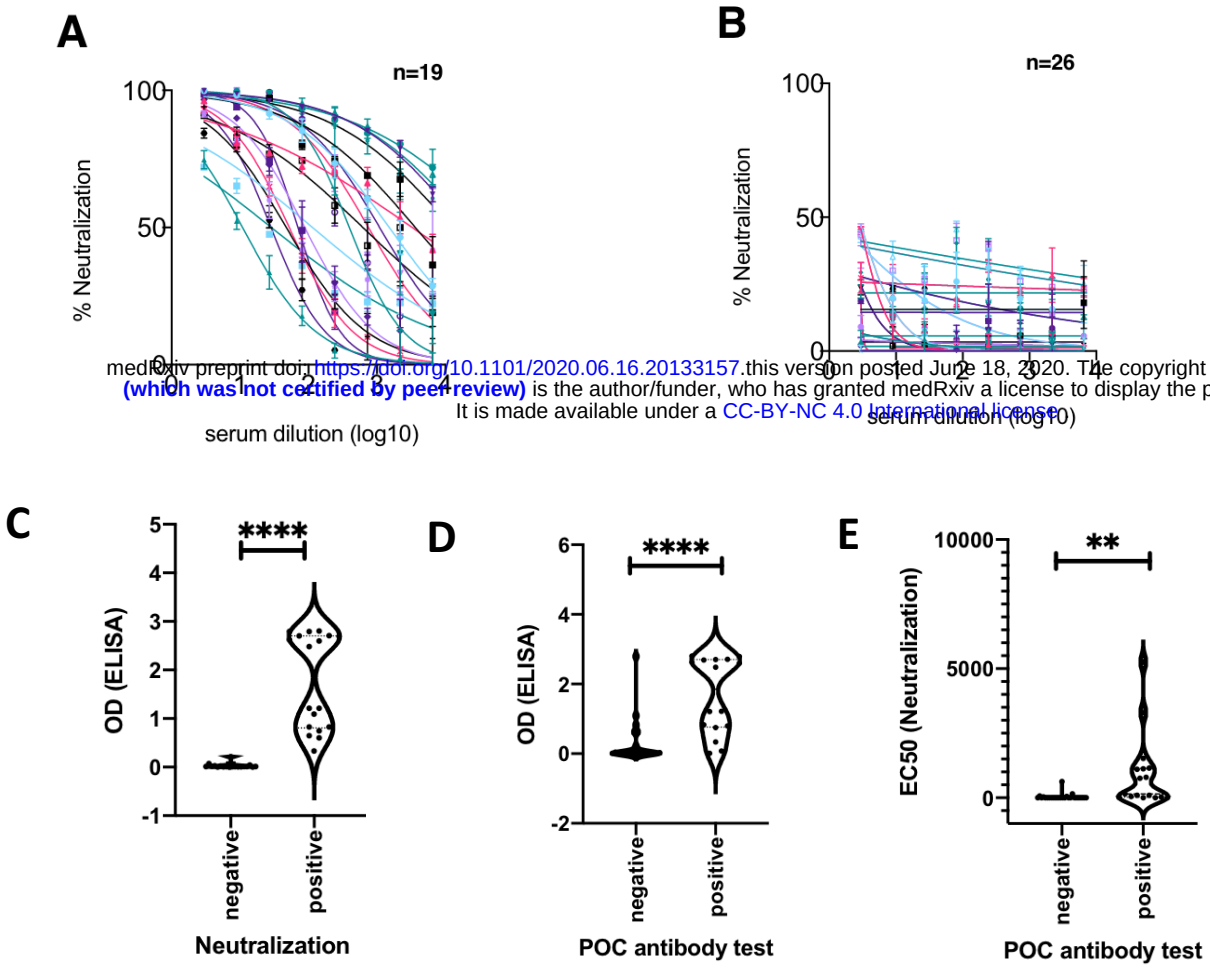

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Figure 1: Antibody detection for SARS-CoV-2: cross validation of a lateral flow diagnostic test (POC antibody test) with ELISA and SARS-CoV-2 pseudotype virus neutralisation assays. A, B. Serum from COVID-19 suspected participants inhibited (n=19) (A) or did not inhibit (n=26) (B) SARS-CoV-2 pseudotype virus infection in a neutralisation assay. Serum from a healthy donor was used and a negative control. The assay was performed in duplicate. Error bars represent SEM. C. Comparison between ELISA and positive/negative results from neutralisation assay. n=37, $p<0.0001$. D. Comparison between ELISA Spike protein reactivity and positive/negative POC antibody test results (COVIDIX SARS-CoV-2 IgM IgG Test). n=38, $p<0.0001$. E. Comparison between EC50 dilution titre from neutralizing assay and positive/negative POC antibody test results (COVIDIX SARS-CoV-2 IgM IgG Test). n=44, $p=0.0025$.

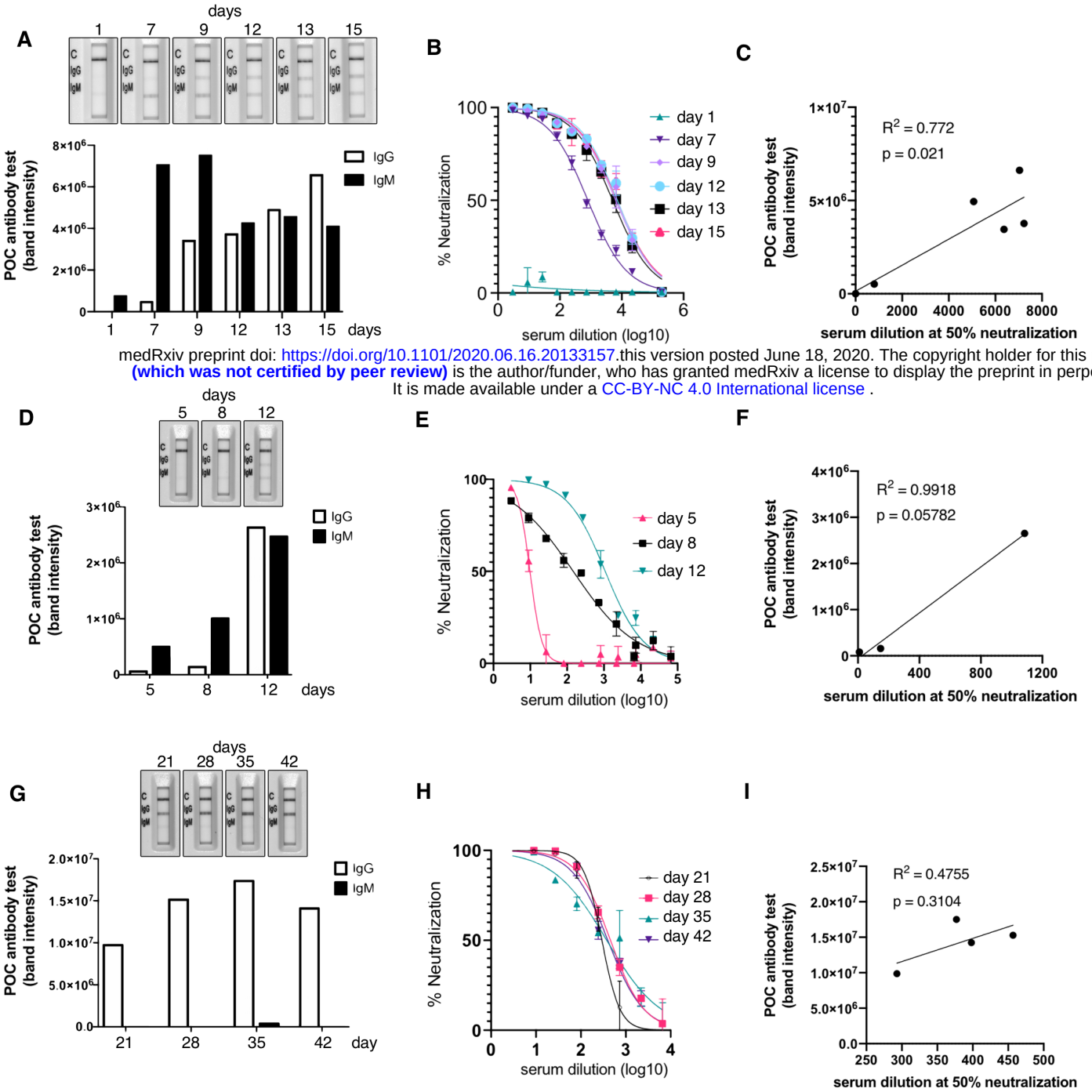

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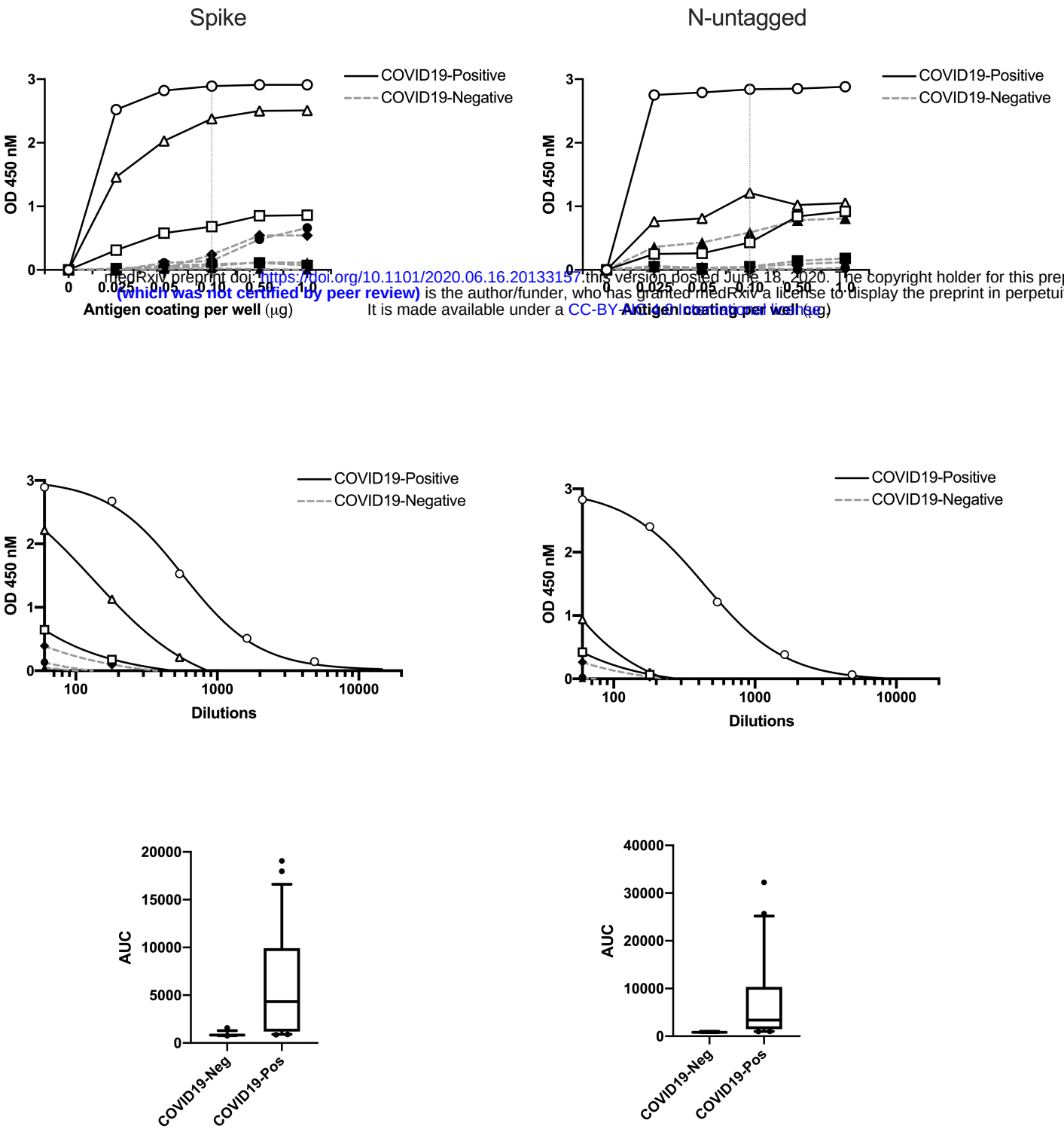
Figure 2: Longitudinal antibody responses detected by rapid lateral flow and neutralisation assays. A, D, G. An immune-chromatographic lateral flow rapid diagnostic test (POC antibody test - COVIDIX SARS-CoV-2 IgM IgG Test) on longitudinal samples in individual patients detecting SARS-CoV-2 IgM and IgG bands. Band intensities were acquired using ChemiDoc MP Imaging System and quantified using Image Lab software. B, E, H. SARS-CoV-2 pseudotyped virus neutralisation assay from longitudinal serum samples in individual patient examples. The assays were performed in duplicate. Error bars represent SEM. C, F, I. Comparison of IgG band intensities from lateral flow rapid diagnostic test with EC50 neutralisation titres from SARS-CoV-2 pseudotyped virus neutralisation assay in individual patients. Correlations were estimated by linear regression analysis.



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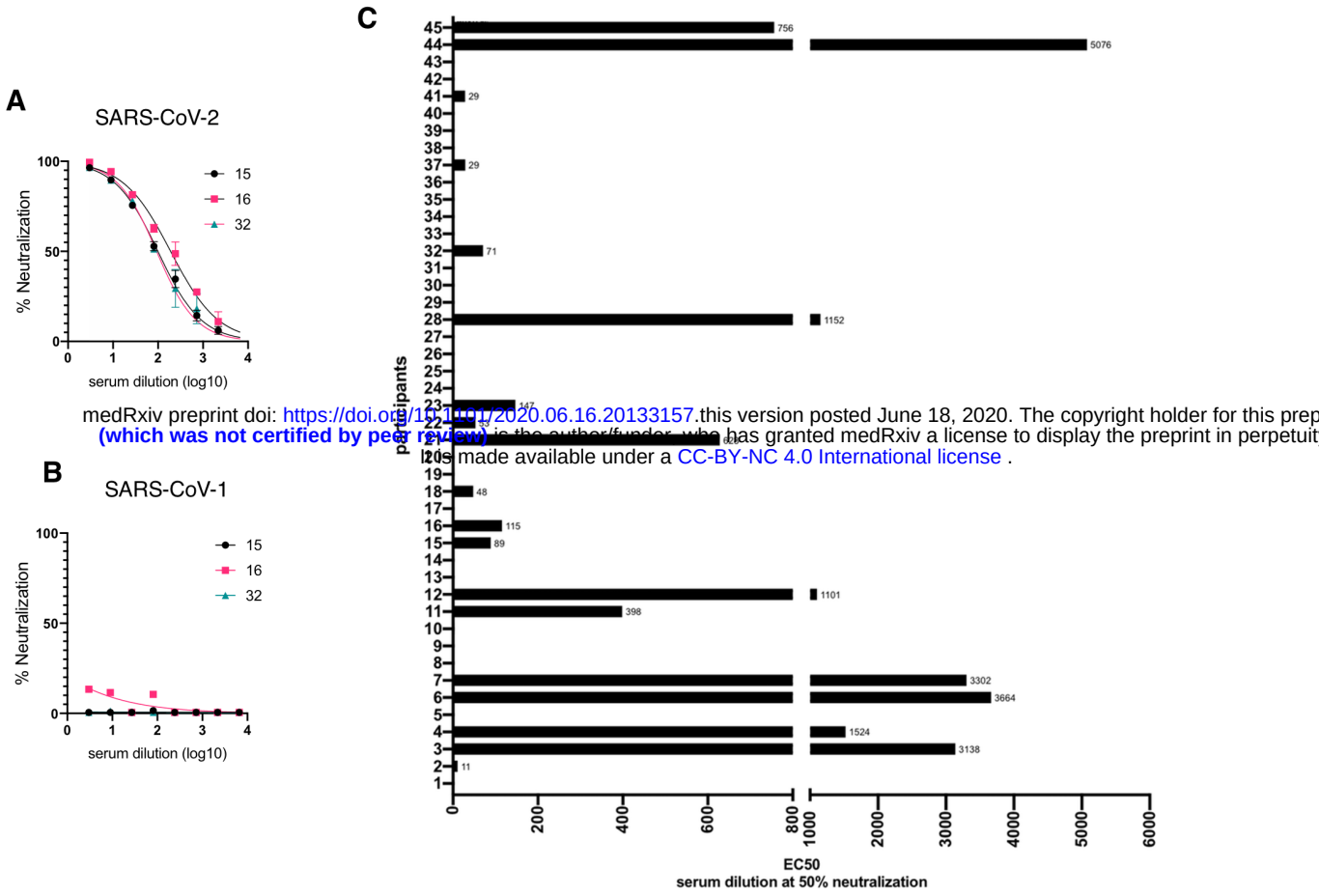
Supplementary Figure 1: Establishment of serological assay to determine positivity and endpoint titre against human SARS CoV-2. Residual stored serum samples from PCR positive and negative patient cohort were screened for reactivity against full-length spike and N-proteins. A) To determine the appropriate concentration of antigen used for plate coating, 0, 0.025, 0.05, 0.1, 0.5 and 1.0 1mg antigen per well was coated and reactivity of known seropositive and seronegative serum samples were examined. B) Subsequently, end-point titrations were performed using 0.1mg per well spike and N antigen coating. C. The area under the curve (AUC) was calculated for every sample using end point titrations against spike (n=76) and N protein (n=64), and the mean and the 95% confidence intervals are shown for all PCR positive and negative samples. OD: optical density (nanometers)



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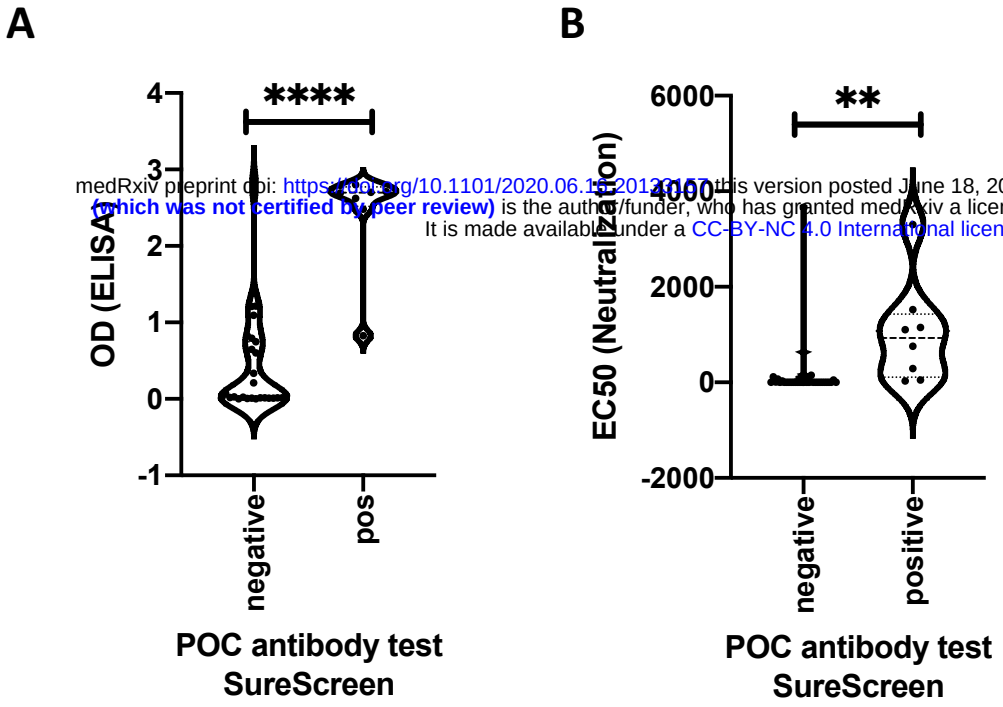
Supplementary Figure 2: Specify of antibody neutralizing response against SARS-CoV-2 and CoV-1.

SARS-CoV-2 **(A)** or SARS-CoV-1 **(B)** Spike protein pseudotyped viral particles were incubated with serial dilutions of heat inactivated human serum samples from Covid-19 suspected individuals (#15,16,32) in duplicates for 1h at 37˚C. 293T ACE2/TMPRSS2 expressing cells were added to each well. Following 48h incubation in a 5% CO2 environment at 37°C, the luminescence was measured using Steady-Glo Luciferase assay system (Promega). Percentage of neutralization was calculated with non-linear regression, log (inhibitor) vs. normalized response using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA). **(C)** The 50% inhibitory dilution (EC50) was defined as the serum dilution at which the relative light units (RLUs) were reduced by 50% compared with the virus control wells (virus + cells) after subtraction of the background RLUs in the control groups with cells only. The EC50 values were calculated with non-linear regression, log (inhibitor) vs. normalized response using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA).



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Supplementary Figure 3: Comparison of a lateral flow diagnostic test (SureScreen SARS-CoV-2 IgM/IgG test) against ELISA IgG and SARS-CoV-2 pseudotype virus neutralisation assays on sera from patients with suspected moderate to severe COVID-19. (A) Comparison between ELISA IgG and positive/negative POC IgG band results. n=38, p<0.0001. (B). Comparison between EC50 dilution titre from neutralisation assay and positive/negative POC IgG antibody band test results. n=43, p=0.005.