Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel [version 1; peer review: 1 approved]

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Abstract
Background: The COVID-19 pandemic caused >1 million infections during January-March 2020. There is an urgent need for reliable antibody detection approaches to support diagnosis, vaccine development, safe release of individuals from quarantine, and population lock-down exit strategies. We set out to evaluate the performance of ELISA and lateral flow immunoassay (LFIA) devices.

Methods: We tested plasma for COVID (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) IgM and IgG antibodies by ELISA and using nine different LFIA devices. We used a panel of plasma samples from individuals who have had confirmed COVID infection based on a PCR result (n=40), and pre-pandemic negative control samples banked in the UK prior to December-2019 (n=142).

Results: ELISA detected IgM or IgG in 34/40 individuals with a confirmed history of COVID infection (sensitivity 85%, 95%CI 70-94%), vs. 0/50 pre-pandemic controls (specificity 100% [95%CI 93-100%]). IgG levels were detected in 31/31 COVID-positive individuals tested ≥10 days after symptom onset (sensitivity 100%, 95%CI 89-100%). IgG titres rose during the 3 weeks post symptom onset and began to fall by 8 weeks, but remained above the detection threshold. Point estimates for the sensitivity of LFIA devices ranged from 55-70% versus RT-PCR and 65-85% versus ELISA, with specificity 95-100% and 93-100% respectively. Within the limits of the study size, the performance of most LFIA devices was similar.

Conclusions: Currently available commercial LFIA devices do not perform sufficiently well for individual patient applications. However, ELISA can be calibrated to be specific for detecting and quantifying SARS-CoV-2 IgM and IgG and is highly sensitive for IgG from 10 days following first symptoms.

Keywords
COVID-19, SARS-CoV-2, serology, IgG, IgM, antibodies, immunoassay, ELISA, lateral flow, exposure, epidemiology

This article is included in the Coronavirus (COVID-19) collection.
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Competing interests: RC reports personal fees and other from MIROBIO Ltd, outside the submitted work. DWE reports personal fees from Gilead, outside the submitted work. SH reports grants from NIHR, during the conduct of the study. AJP reports grants from NIHR Oxford Biomedical Research Centre, outside the submitted work; and AJP is Chair of UK Dept. Health and Social Care’s (DHSC) Joint Committee on Vaccination & Immunisation (JCVI) and is a member of the WHO's SAGE. The views expressed in this article do not necessarily represent the views of DHSC, JCVI, NIHR or WHO. GRS reports personal fees from GSK Vaccines SAB. MGS reports grants from National Institute of Health Research, grants from Medical Research Council UK, grants from Health Protection Research Unit in Emerging & Zoonotic Infections, University of Liverpool, during the conduct of the study; other from Integrum Scientific LLC, Greensboro, NC, USA, outside the submitted work. ASW reports grants from NIHR, during the conduct of the study. No other author has a conflict of interest to declare.

Grant information: This study was supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre, the UK Government Department of Health and Social Care and grants from NIHR [award CO-CIN-01] and the Medical Research Council [grant MC_PC_19059]. DC, TEAP and ASW are supported by the National Institute for Health Research (NIHR) Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance at the University of Oxford in partnership with Public Health England (NIHR200915). Blood donor and QUOD samples were provided with support from NHS Blood and Transplant and the Medical Research Council UK. SKC is supported by Medical Research Council UK. Tds is funded by a Welcome Trust Intermediate Clinical Fellowship (110058). DWE is a Robertson Foundation Fellow and NIHR Oxford BRC Senior Research Fellow. PCM is a Wellcome Trust Clinical Research Fellow (110110) and NIHR Oxford BRC Senior Research Fellow. LT is supported by a Wellcome clinical career development fellowship (205228/Z/16/Z), EA, AM, PK, SCM, TPR, MGS and LT are supported by NIHR Health Protection Research Unit in Emerging and Zoonotic Infections (HPRU-EZI) at University of Liverpool in partnership with Public Health England (PHE), in collaboration with the University of Oxford and Liverpool School of Tropical Medicine (award number NIHR200907). EB, PK, AJP and ASW are NIHR Senior Investigators. PK (WT109965/MA) and GRS (095541) are Wellcome Trust Senior Investigators. JR is supported by a Sir Henry Dale Fellowship, jointly funded by the Wellcome Trust and the Royal Society (Grant 101237). The views expressed are the author(s) and are not necessarily those of the NHS, the NIHR, the UK Department of Health and Social Care, the MRC or PHE.

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How to cite this article: Adams ER, Ainsworth M, Anand R et al. Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel [version 1; peer review: 1 approved] Wellcome Open Research 2020, 5:139
https://doi.org/10.12688/wellcomeopenres.15927.1
First published: 11 Jun 2020, 5:139 https://doi.org/10.12688/wellcomeopenres.15927.1
Introduction
The first cases of infection with a novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) causing coronavirus infectious disease (COVID) emerged in Wuhan, China on December 31st, 2019. Despite intensive containment efforts, there was rapid international spread and three months later, there had been over 1 million confirmed infections and 60,000 reported deaths. Containment efforts have relied heavily on population quarantine (‘lock-down’) measures to restrict movement and reduce individual contacts. To develop public health strategies for exit from lock-down, diagnostic testing urgently needs to be scaled-up, including both mass screening and screening of specific high-risk groups (contacts of confirmed cases, and healthcare workers and their families), in parallel with collecting data on recent and past infection at individual and population levels.

Laboratory diagnosis of infection has mostly been based on real-time RT-PCR, typically targeting the viral RNA-dependent RNA polymerase (RdRp) or nucleocapsid (N) genes using swabs collected from the upper respiratory tract. This requires specialist equipment, skilled laboratory staff and PCR reagents, creating diagnostic delays. RT-PCR from upper respiratory tract swabs may also be falsely negative due to quality or timing; viral loads in upper respiratory tract secretions peak in the first week of symptoms, but may have declined below the limit of detection in those presenting later. In individuals who have recovered, RT-PCR provides no information about prior exposure or immunity.

In contrast, assays that reliably detect antibody responses specific to SARS-CoV-2 could contribute to diagnosis of acute infection (via rises in IgM and IgG levels) and to identifying those infected with or without symptoms and recovered (via persisting IgG). Receptor-mediated viral entry to host cells occurs through interactions between the unique and highly-conserved viral spike (S) glycoprotein and the ACE2 cell receptor. This S protein is the primary target of specific neutralising antibodies, and current SARS-CoV-2 serology assays therefore typically seek to identify these antibodies (Figure 1A–C). Rapid lateral flow immunoassay (LFIA) devices provide a quick, point-of-care approach to antibody testing. A sensitive and specific antibody assay could directly contribute to early identification and isolation of cases, address unknowns regarding the extent of infection to inform mathematical models and support individual or population-level release from lock-down. Laboratory-based ELISA platforms have also been evaluated as an approach to detection and quantification of SARS-CoV-2 antibodies.

However, before either laboratory assays or LFIA devices can be widely deployed, their performance needs to be carefully evaluated (Figure 1D, E). We therefore compared a novel laboratory-based ELISA assay with nine commercially-available LFIA devices using samples from patients with RT-PCR-confirmed infection, and negative pre-pandemic samples.

Methods
Research reporting
Samples. A total of 142 plasma samples designated seronegative for SARS-CoV-2 were collected from adults (≥18 years) in the UK before December 2019 (Underlying data, Table S1, including demographic details) from three ethical approved sources: healthy blood donors, organ donors on ICU following cerebral injury and healthy volunteers from a vaccine study.

In total, 40 plasma samples were collected from adults positive for SARS-CoV-2 by RT-PCR from an upper respiratory tract (nose/throat) swab tested in accredited laboratories (Underlying data, Table S1). Acute (≤28 days from symptom onset) and convalescent samples (>28 days) were included to optimise detection of SARS-CoV-2 specific IgM and IgG respectively (Figure 1B). Acute samples were collected from patients a median 10 (range 4–27) days from symptom onset (n=16), and from recovering healthcare workers median 13 [range 8–19] days after first symptoms; (n=6). Convalescent samples were collected from adults a median 48 [range 31–62] days after symptom onset and/or date of positive throat swab (n=18). Further sample details are provided in Extended data, Supplementary Material.

Cases were classified following WHO criteria as critical (respiratory failure, septic shock, and/or multiple organ dysfunction/failure); severe (dyspnoea, respiratory frequency ≥30/minute, blood oxygen saturation ≤93%, PaO2/FiO2 ratio <300, and/or lung infiltrates >50% of the lung fields within 24–48 hours); or otherwise mild. Among 22 acute cases, 9 were critical, 4 severe and 9 mild. All but one convalescent individual had mild disease; the other was asymptomatic and screened during enhanced contact tracing.

ELISA
We developed a novel ELISA targeting the SARS-CoV-2 spike protein. Recombinant SARS-CoV-2 trimeric spike protein was constructed as described, using mammalian codon optimised SARS2 Spike (1–1208, Genbank accession MN908947) with a GSAS substitution at the furin cleavage site (aa 682–685) and double proline substitution at aa 986–987. The C-terminal was followed by T4 fibritin motif, an HRV3C protease cleavage site, a TwinStrep Tag and an 8-HisTag. The gene was cloned into a pHSec and expressed in 293T cells. The HIS trap HP column (cat no 17524701; Cytiva) was used to purify the recombinant S protein.

We used ELISA to detect antibodies to the S protein. MAXISORP immunoplates (442404; NUNC) were coated with StreptMAB-Classic (2-1507-001;iba). Plates were blocked with 2% skimmed milk in PBS for one hour and then incubated with 0.125 μg of soluble trimeric SARS-CoV-2 trimeric S protein or 2% skimmed milk in phosphate buffered saline. After one hour, plasma was added at 1:50 dilution, followed by ALP-conjugated anti-human IgG (A9544, RRID:AB_258459; Sigma) at 1:10,000 dilution or ALP-conjugated anti-human IgM (A9794, RRID:AB_258474; Sigma) at 1:5,000 dilution. The reaction was developed by the addition of PNPP substrate and stopped with 1.0 M NaOH. The absorbance was measured at 405nm after 90 minutes, and a final optical density (OD) value was calculated by subtracting the background (skimmed milk) from the test value. The ELISA assay takes 5–6 hours to perform with an experienced operator being able to process up to five 96-well plates (480 samples including relevant controls).
**Figure 1.** Cartoon to illustrate the generation of IgM and IgG antibodies to SARS nCoV-2 and detection of antibodies by a lateral flow device. (A) **In vivo** generation of antibodies to the trimeric SARS-CoV-2 spike protein. (B) Projected change in titres of specific IgM and IgG over time following infection, with arrows indicating typical time frames for collection of acute and convalescent samples. (C) **Ex vivo** detection of IgG and/or IgM using a lateral flow immunoassay (LFIA): S= sample well, T=test antibody; C=control. Diagram shows a positive sample on the left, with positive lines at both C and T, and a negative test on the right with a line present only at C. Any other combination of lines renders the test invalid. Some devices have two test lines, for separate detection of anti-SARS-CoV-2-IgG and -IgM. Assays variably suggest use of plasma, serum and/or whole blood. (D) Outcomes of testing negative and positive samples using LFIA. (E) Calculation of sensitivity, specificity, positive and negative predictive value of a test. Image created with BioRender.com; exported under a paid subscription.

**LFIA**

We tested LFIA devices designed to detect IgM, IgG or total antibodies to SARS-CoV-2 produced by nine manufacturers short-listed as a testing priority by the UK Government Department of Health and Social Care (DHSC), based on appraisals of device provenance and available performance data. Individual manufacturers did not approve release of device-level data, so device names are anonymised.

Testing was performed in strict accordance with the manufacturer’s instructions for each device. Typically, this involved adding 5–20 µl of plasma to the sample well, and 80–100 µl of manufacturer’s buffer to an adjacent well, followed by incubation at room temperature for 10–15 minutes. The result was based on the appearance of coloured bands, designated as positive (control and test bands present), negative (control band only), or invalid (no band, absent control band, or band in the wrong place) (Figure 1C).

We recorded results in real-time on a password-protected electronic database, using pseudonymised sample identifiers, capturing the read-out from the device (positive/negative/invalid), operator, device, device batch number, and a timestamped photograph of the device.

**Testing protocol**

We tested 90 samples using ELISA to quantify IgM and IgG antibody in plasma designated SARS-CoV-2 negative (n=50) and positive (n=40). All positive samples were included and an unstratified random sample of negative plasma from healthy blood
donors (n=23) and organ donors (n=27). We tested the nine different LFIA devices using between 39–165 individual plasma samples (8–23 and 31–142 samples designated SARS-CoV-2 positive and negative, respectively, Table S2). Total numbers varied according to the number of devices supplied to the DHSC; samples were otherwise selected at random.

Statistical analysis
Analyses were conducted using R (version 3.6.3) and Stata (version 15.1), with additional plots generated using GraphPad Prism (version 8.3.1). Binomial 95% confidence intervals (CI) were calculated for all proportions. The association between ELISA results and 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. Differences between LFIA devices were estimated using mixed effects logistic regression models, allowing for each device being tested on overlapping sample sets. Differences between devices were compared with Benjamini-Hochberg corrected p-value thresholds. (Further details in Extended data, Supplementary Material.)

Ethical approval and role of the funding source
Our work was undertaken with ethical approval from the National Health Service Blood and Transplant (NHSBT) ethics, providing donor consent for plasma use; NIHR Biobank REC agreement (REC 13/NW/0017; IRAS 87824); International Severe Acute Respiratory and Emerging Infection Consortium (‘ISARIC’) approval by the South Central (Oxford C) Research Ethics Committee in England (Ref: 13/SC/0149), and Scotland A Research Ethics Committee in Scotland (Ref: 20/SS/0028). The UK Government DHSC selected the lateral flow devices for testing as described above. Otherwise, the funders had no role in study design or in the collection, analysis, and interpretation of data. Authors from DHSC contributed to writing of the report and in the decision to submit the paper for publication.

An earlier version of this article can be found on medRxiv (DOI: https://doi.org/10.1101/2020.04.15.20066407).

Results
Detection of SARS-CoV-2 IgM and IgG antibody by ELISA
The 40 positive (RT-PCR-confirmed SARS-CoV-2 infection) and 50 designated negative (pre-pandemic) plasma samples were tested by ELISA to characterise antibody profiles. Negative samples had median optical density (OD) for IgM of -0.0001 (arbitrary units) (range -0.14 to 0.06) and for IgG -0.01 (range -0.38 to 0.26). The median IgM reading in 40 positive samples was 0.18 (range -0.008 to 1.13; Kruskal-Wallis p<0.001 vs. negative) and IgG median 3.0 (range -0.2 to 3.5; p<0.001).

As safe individual release from lock-down is a major application for serological testing, we chose OD thresholds that maintained 100% specificity (95%CI 93–100%), while maximising sensitivity. Using thresholds of 0.07 for IgM and 0.4 for IgG (3 and 5 standard deviations above the negative mean, respectively; Figure 2A, B), the IgG assay had 85% sensitivity (95%CI 70–94%; 34/40) vs. RT-PCR diagnosis. All six false negatives were from samples taken within 9 days of symptom onset (Figure 2D). IgG levels were detected in 31/31 RT-PCR-positive individuals tested ≥10 days after symptom onset (sensitivity 100%, 95%CI 89–100%). The IgM assay sensitivity was lower at 70% (95%CI 53–83%; 28/40). All IgG false-negatives were IgM-negative. No sample was IgM-positive and IgG-negative.

Considering the relationship between IgM and IgG titres and time since symptom onset (Figures 2C, D), univariable regression models showed IgG antibody titres rising over the first 3 weeks from symptom onset. The lower bound of the pointwise 95%CI for the mean expected titre crosses our OD threshold between days 6–7 (Figure 2D). However, given sampling variation, test performance is likely to be optimal from several days later. IgG titres fell during the second month after symptom onset but remained above the OD threshold. No temporal association was observed between IgM titres and time since symptom onset (Figure 2C). There was no evidence that SARS-CoV-2 severity, need for hospital admission or patient age were associated with IgG or IgM titres in multivariable models (p>0.1, Table S3).

Detection of SARS-CoV-2 antibodies by LFIA vs. RT-PCR
We first considered performance of the nine different LFIA devices using RT-PCR-confirmed cases as the reference standard (Table 1A and Extended data, Figure S1) and considering any LFIA positive result (IgM, IgG or both) as positive. The LFIA devices achieved sensitivity ranging from 55% (95%CI 36–72%) to 70% (51–84%) and specificity from 95% (95%CI 86–99%) to 100% (94–100%). There was no evidence of differences between the devices in sensitivity (p>0.1, cf. Benjamini-Hochberg p=0.0014 threshold) or specificity (p=0.19 for all devices with at least one false-positive test). Restricting to 31 samples collected ≥10 days post symptom-onset (all ELISA IgG-positive), LFIA sensitivity ranged from 61% (95%CI 39–80%) to 88% (68–97%) (Extended data, Table S4).

Detection of SARS-CoV-2 antibodies by LFIA vs. ELISA
We also considered performance relative to ELISA (Extended data, Table S5, Figure S1), because the LFIA devices target the same antibodies. We considered patients positive by this alternative standard if their IgG OD reading exceeded the threshold described above, since no samples were IgM-positive, IgG-negative. Sensitivity of antibody detection by LFIA ranged from 65% (95%CI 46–80%) to 85% (66–96%) and specificity from 93% (95%CI 83–98%) to 100% (94–100%); however, the device with the highest sensitivity had one of the lowest specificities (Extended data, Figure S1). There was no evidence of differences in sensitivity (p>0.010, cf. p=0.0014 threshold) or specificity between devices (p>0.19).

Of 50 designated negative samples tested by both ELISA and the nine different LFIA devices, nine separate samples generated at least one false-positive, on seven different LFIA devices (Figure 3). Four samples generating false-positive results did so on more than one LFIA device, despite the absence of
Figure 2. Results of testing 90 plasma samples for SARS-CoV-2 IgM and IgG by Enzyme linked Immunosorbent Assay (ELISA). (A) IgM readings for SARS-CoV-2 pre-pandemic plasma (designated negatives, shown in blue, n=50), and RT-PCR confirmed cases of SARS-CoV-2 infection (designated positives, shown in orange, n=40; divided into acute cases, n=22, and convalescent cases, n=18. Threshold of OD = 0.07 discriminates accurately between negative controls and convalescent sera. (B) IgG data shown for the same subgroups described for panel (A). A threshold of OD = 0.4 discriminates between designated negatives and positives. (C) IgM OD values plotted against the time post symptoms at which plasma was obtained. The line shows the mean OD value expected from a spline-based linear regression model, the ribbon indicates the pointwise 95% confidence interval. (D) IgG OD values plotted against the time post symptoms at which plasma was obtained. Coloured dots in panels C and D indicate disease severity. OD = optical density.
quantifiable IgM or IgG on ELISA, potentially suggesting a specific attribute of the sample causing a cross-reaction on certain LFIA platforms.

Of the 22 samples collected from RT-PCR positive patients in the acute setting, six fell below the ELISA detection threshold for IgM or IgG; two of these six were positive on LFIA testing, each on one (different) device. Of the remaining 16 acute samples (all ELISA IgG-positive), only nine were consistently positive across all nine LFIA devices. Due to limited availability of LFIA devices, fewer tests were performed on the 18 convalescent samples with available ELISA data, all with quantifiable IgG. Two had no antibody detected on any LFIA device, and only eight were consistently positive across all LFIA devices tested (between 1 and 9 devices tested per sample). Full metadata for results of ELISA and LFIA devices are available in Underlying data, Supplementary Table S6.

**Discussion**

We here present the performance characteristics of a novel ELISA and nine selected LFIA devices for detecting SARS-CoV-2 IgM and IgG. Among 40 RT-PCR-confirmed positive patients, 85% had IgG detected by ELISA, including 100% patients tested ≥10 days after symptom onset. A panel of LFIA devices had sensitivity between 55 and 70% against the reference-standard RT-PCR, or 65–85% against ELISA, with specificity of 95–100% and 93–100%, respectively. These estimates come with wide confidence intervals due to constraints on the number of devices made available. Comparable results have been obtained through a similar appraisal undertaken independently, in which specificity ranged from 84–100.0%, and the proportion of specimens testing positive increased over time from symptom onset, with >80% sensitivity achieved by some LFIA devices at later time points. Our study, and these parallel data from another centre, provide a benchmark against which to assess the performance of future antibody testing platforms, with the aim of guiding decisions about deploying antibody testing and informing the design of second-generation assays.

LFIA devices are cheap to manufacture, store and distribute, and could be used as a point-of-care test, offering an appealing approach to diagnostics and evaluating exposure, were adequate performance to be confirmed. A positive antibody test is currently regarded as a probable surrogate for immunity to reinfection. Secure confirmation of antibody status would therefore reduce anxiety, provide confidence to allow individuals to relax social distancing measures, and guide policy-makers in the staged release of population lock-down, potentially in tandem with digital approaches to contact tracing. As a diagnostic tool, serology may have a role in combination with RT-PCR testing to improve sensitivity, particularly of cases presenting some time after symptom onset. Reproducible methods to detect and quantify vaccine-mediated antibodies are also crucial as COVID vaccines enter clinical trials.

Appropriate thresholds for sensitivity and specificity depend on the primary purpose of the test. For diagnosis in symptomatic patients, high sensitivity is required (generally ≥90%). Specificity

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**Table 1. Results of nine lateral flow immunoassays (LFIA) devices and an ELISA assay, tested with plasma classified as positive (RT-PCR positive) and negative (pre-pandemic).**

<table>
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Figure 3. Comparison between ELISA and LFIA for SARS-CoV-2 designated negative and positive plasma. (A) Quantitative optical density (OD) readout from ELISA for IgG for designated negative plasma (n=50) and from individuals with RT-PCR confirmed infection (n=40, divided into acute and convalescent plasma). IgM results are shown in Extended data, Figure S2. (B) Results from LFIA produced by nine manufacturers. Any positive test for IgG, IgM, both or total antibody is shown as positive; see Extended data, Figure S2 for more detailed breakdown. 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 both panels are ranked from left to right by quantitation of IgG, as indicated in panel (A).

Appraisal of test performance should also consider the influence of population prevalence, acknowledging that this changes over time, geography and within different population groups. The potential risk of a test providing false reassurance and release from lock-down of non-immune individuals can be considered as the proportion of all positive tests that are wrong. Based on the working ‘best case’ scenario of a LFIA test with 70% sensitivity and 98% specificity, the proportion of positive tests that are wrong is 35% at 5% population seroprevalence (19 false-positives/1000 tested), 10% at 20% seroprevalence (16 false-positives/1000) and 3% at 50% seroprevalence (10 false-positives/1000) (Figure 4).

More data are needed to investigate antibody-positivity as a correlate of protective immunity. Indeed, pre-existing IgG could enhance disease in some situations, with animal data demonstrating that SARS-CoV-2 anti-spike IgG contributes to a proinflammatory response associated with lung injury in macaques.
Figure 4. Influence of population prevalence of seropositivity on assay performance. Scenarios with population prevalence of 5%, 20% and 50% are shown within each panel. (A) The proportion of all positive tests that are wrong (1-positive predictive value), which would lead to false release from lock-down of non-immune individuals, for varying test sensitivity (x-axis) and 1-specificity (line colour). (B) The proportion of negative tests that are wrong. (C) The absolute number of false positive tests per 1000 tests. (D) The absolute number of false negative tests per 1000 tests.

Our study, and another undertaken independently in parallel\textsuperscript{11}, demonstrates accurate performance of ELISA targeting anti-spike protein antibodies. Additionally, our ELISA results are supported by context regarding disease severity and the time of sampling relative to symptom onset. Our data on the kinetics of antibody responses build upon studies of hospitalised patients in China reporting a median 11 days to seroconversion for total antibody, with IgM and IgG seroconversion at days 12 and 14, respectively\textsuperscript{18}, and others that report 100% IgG positivity by 15–19 days\textsuperscript{19,23}. Our ELISA data show IgG titres rose over the first 3 weeks of infection and that IgM testing identified no additional cases. Methods to enhance sensitivity, especially shortly after symptom onset, could consider different sample types (e.g. saliva), different antibody classes (e.g. IgA)\textsuperscript{24}, T-cell assays or antigen detection\textsuperscript{25}. In contrast to others\textsuperscript{26–28}, we did not find evidence of an association between disease severity and antibody titres. We observed several LFIA false positives, which may have potentially resulted from cross-reactivity of non-specific antibodies (e.g. reflecting past exposure to other seasonal coronavirus infections).

The main study limitation is that numbers tested were too small to provide tight confidence intervals around performance estimates for any specific LFIA device. Expanding testing across diverse populations would increase certainty, but given the broadly comparable performance of different assays, the cost and manpower to test large numbers may not be justifiable. Demonstrating high specificity is particularly challenging; for example, if the true underlying value was 98%, 1000 negative controls would be required to estimate the specificity of an assay to ±1% with approximately 90% power. Full assessment should also include a range of geographical locations and ethnic groups, children, and those with immunological disease including autoimmune conditions and immunosuppression.

In summary, antibody testing is a crucial component of measures that may be required to inform release from lockdown. Our findings suggest that while current LFIA devices may provide some information for population-level surveys, their performance is inadequate for most individual patient applications. The ELISA we describe is currently being optimised.
and adapted to run on a high-throughput platform and provides promise for the development of reliable approaches to antibody detection that can support decision making for clinicians, the public health community, policy-makers and industry.

Data availability
Underlying data

This project contains the following underlying data:
- Supplementary_table_s1 (XLSX). (Metadata describing origin and characteristics of designated negative controls and individuals with confirmed SARS-CoV-2 infection.)
- Supplementary_table_s6 (XLSX). (Results of all assays performed and relevant metadata.)

Extended data

File ‘Supplementary material’ (PDF) contains the following extended data:
- Supplementary methods.
- 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).
- Figure S2. Comparison between ELISA and LFIA for SARS-CoV-2 designated negative and positive plasma.
- Supplementary table S2. Summary grid presenting the number of samples from each cohort tested using different assay platforms.
- Supplementary table S3. Multivariable regression models for relationship between ELISA IgM and IgG readings and covariates in RT-PCR positive cases.
- Supplementary table S4. Results of nine LFIA devices and an ELISA assay, tested with plasma classified as positive (RT-PCR positive) obtained from patients ≥10 days after onset of symptoms.
- Supplementary table S5. Results of nine LFIA devices, tested with plasma classified as positive and negative using ELISA as an alternative reference standard.

Reporting guidelines

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgements
This work uses data and samples provided by patients and collected by the NHS as part of their care and support. We are extremely grateful to the frontline NHS clinical and research staff and volunteer medical students, who collected this data in challenging circumstances; and the generosity of the participants and their families for their individual contributions. We would like to thank Carla Wright and Rosie McMahon for administrative assistance.

Author information
The members and affiliations of the National COVID Testing Scientific Advisory Panel are listed in the author list, in alphabetical order.

Author contributions
Leadership roles were undertaken by DC (project management), RJP and DJR (management of pre-pandemic negative sample bank), AJM (identification and recruitment of cases), DWE and PCM (oversight of LFIA, data analysis and manuscript writing), GRS (ELISA design, development and data generation).

References


Open Peer Review

Current Peer Review Status: ✔️

Version 1

Reviewer Report 15 September 2020

https://doi.org/10.21956/wellcomeopenres.17469.r40226

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This is a systematic study that compares a laboratory-based home-made ELISA serological assay for COVID-19 with nine commercially-available rapid lateral flow immunoassay (LFIA). The results presented are convincingly and important for the development/improvement of serological assays for COVID-19. It will be helpful if the authors can clarify the following points:

1. Page 4, Methods: 2nd paragraph, line 4: Acute (28 days).
   Query: How did the authors decide on the 28 days cut-off? Is this definition similar to other studies on COVID-19 seroconversion? It would be good to discuss and include citation.

2. Page 6, it was stated “We also considered performance relative to ELISA (Extended data, Table S5, Figure S1), because the LFIA devices target the same antibodies.”
   Query: The viral protein used each of the LFIA device is not stated in the manuscript. Based on this sentence, are the authors stating that all of them use SARS-CoV-2 Spike residues 1-1208? If not, please state the viral protein or fragment used for each device.

3. Page 9, “... with animal data demonstrating that SARS-CoV-2 anti-spike IgG contributes to a pro-inflammatory response associated with lung injury in macaques.”
   Query: SARS-CoV-2 should be SARS-CoV.

4. Page 10, it was stated “We observed several LFIA false positives, which may have potentially resulted from cross-reactivity of non-specific antibodies (e.g. reflecting past exposure to other seasonal coronavirus infections).
   Query: Are these LFIA using the same viral protein or fragment? What is the sequence
homology between SARS-CoV-2 and endemic coronaviruses for each of viral protein used?

5. Page 10, it was stated “In contrast to others, we did not find evidence of an association between disease severity and antibody titres”
Query: Is there any difference in study design between this study and the published studies cited? Were the published studies measuring anti-Spike binding antibodies or were they measuring neutralizing antibodies? Are the sample size and distribution of cases (mild vs severe, etc) comparable? Please highlight any limitation that may explain the discrepancy.

6. For the simulations shown in Figure 4.
Query: Please provide details on method/program as well as assumptions used.

7. The ELISA described by the authors is important and adds value to the development of serological assay for COVID-19. To briefly show the robustness of the assay, can authors provide information on (a) intra-assay variability, (b) inter-assay variability, (c) inter-operators variations and (d) the turnaround time i.e. from sample to result?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Molecular virology; Monoclonal antibody technology;

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.