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Laboratory evaluation of SARS-CoV-2 antibodies: detectable IgG up to 20 weeks post infection.

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Abstract

Objective

To evaluate the dynamics and longevity of the humoral immune response to SARS-CoV-2 infection and assess the performance of the UK-RTC AbC-19 Rapid Test lateral flow immunoassay (LFIA) for the target condition of SARS-CoV-2 spike protein IgG antibodies.

Design

Nationwide serological study.

Setting

Northern Ireland, UK, May - August 2020.

Participants

Plasma samples were collected from a diverse cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations and research studies (n=223) and through a convalescent plasma program (n=183).

Main Outcome Measures

SARS-CoV-2 antibody levels in plasma samples using Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2 ELISA immunoassays over time. UK-RTC AbC-19 LFIA sensitivity and specificity, estimated using a reference standard system to establish a characterised panel of 330 positive and 488 negative SARS-CoV-2 IgG samples.

Results

We detected persistence of SARS-CoV-2 IgG up to 140 days (20 weeks) post infection, across all three laboratory-controlled immunoassays. On the known positive cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58% (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-99.95%).

Conclusions

Through comprehensive analysis of a cohort of pre-pandemic and pandemic individuals, we show detectable levels of IgG antibodies, lasting up to 140 days, providing insight to antibody levels at later time points post infection. We show good laboratory validation performance metrics for the AbC-19 rapid test for SARS-CoV-2 spike protein IgG antibody detection in a laboratory-based setting.

Strengths and Limitations

Strength - This paper describes for the first time a non-clinical laboratory evaluation and comparison of the ability of three different immunoassays to detect SARS-CoV-2 antibodies in the same samples detecting different subtypes of antibodies against different targets of the viral antigenic repertoire, that does not rely on PCR-positivity as definition of expected test outcome, to provide a panel of known antibody positive and antibody negative serology for evaluation of newly developed immunoassays.

Strength - This study demonstrates AbC-19 lateral flow point of care detection of IgG antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the antibodies made in response to the vaccines used globally, in a large cohort of subjects, 330 positive samples, up to 140 days post infection, across a broad age range (18-90 years). Robust antibody responses were observed in all age groups tested, including over-65s, who are most at risk of severe COVID-19 symptoms, with the eldest in our population having priority for mass vaccination.

Strength - This study shows excellent correlation between approved laboratory-based assays and the newly developed AbC-19 lateral flow point of care lateral flow test for the detection of SARS-CoV-2 antibodies in characterised cohorts of known positive and negative plasma samples in an evaluation conducted according to MHRA guidelines during a pandemic.

Limitation- This study was conducted in a standardised setting with very experienced users on plasma characterised as positive or negative for the presence of antibodies

using a reference standard alongside one other assay which would allow for the possibility of spectrum bias and may well not reflect the true performance metrics of any of the assays evaluated when translated to real life settings, using finger prick blood samples, in which pre-test probability would impact greatly on positive and negative predictive values.

Keywords

SARS-CoV-2, COVID-19, immunoassay, LFIA, pandemic, antibody assay

Introduction

The World Health Organization declared a pandemic in March 2020 due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), identified late 2019 in Wuhan, China, causing COVID-19 disease (1,2).

A global race ensued to develop diagnostic assays, with the most common being viral RNA detection (RT-qPCR assays), to detect acute infection(3). RT-qPCR assays are labour and reagent intensive, limited by a short temporal window for positive diagnosis, and exhibit potential for false negative results (4). Evidence suggests sensitivity of RT-qPCR can be as low as 70% (5). Lockdown measures and "flattening the curve" strategies meant many infected individuals were instructed to self-isolate and were not offered a diagnostic RT-qPCR, with much of the testing limited to patients admitted to hospital, who perhaps reflect a more severely infected cohort. Consequently, a potentially large number of cases were unconfirmed or undetected(6).

The ability to accurately detect SARS-CoV-2 specific antibodies, which develop after an immune response is evoked, is vital for building biobanks of convalescent sera for treatment, monitoring immune response to infection alongside surveillance studies

and assessing responses to vaccination programmes. The timing for when antibody against the novel SARS-CoV-2 virus can be measured is at this time not fully characterised.

Commercial serology immunoassays are mostly laboratory-based and measure IgG antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIAs), require a finger prick blood sample and can be used at point-of-care (POC) or in the home; particularly important in the context of lockdown enforcement during the pandemic. Currently, a limited number of laboratory-based chemiluminescence immunoassays are approved for use in the UK including the Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region (Roche Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay against the same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

The complexities of the humoral immune response to SARS-CoV-2 is a much-debated topic. In a US study, approximately one in 16 individuals lacked detectable IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR confirmed infection (7). Patients who remain asymptomatic may mount a humoral immune response which is short-lived, with detectable levels of antibody falling rapidly (8). This, alongside potentially low sensitivity and lack of RT-PCR test availability across the UK has hindered development of well characterised gold standard serology test for IgG antibodies to SARS-CoV-2.

Herein, we describe the use of Roche and Abbott commercial immunoassays, as well as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike antigenic protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-pandemic and pandemic COVID-19 blood samples (n=880) from within Northern Ireland and report on longevity of IgG antibodies detected. Presently, there is no gold

standard assay for comparison, therefore we aimed to establish a reference based on a positive COVID-19 antibody status. We present results of a laboratory evaluation of the UK-RTC AbC-19 with a target condition of antibodies against a cohort of 330 known IgG antibody positive samples according to this 'positive by two' system and 488 negative samples (223 pre-pandemic assumed negative and 265 known negative) for IgG to SARS-CoV-2.

Methods

Participant samples

The flow of participant samples is summarised in Figure S1. A small cohort (n=19) of anonymised plasma samples were obtained from a partner USA laboratory for initial protocol development only. All participants provided informed consent with no adverse events. An online recruitment strategy was employed, with the study advertised through internal Ulster University email, website and social media. A BBC Newsline feature providing the pandemic study email address also prompted interest from the general population. The first 800 respondents who expressed interest were provided with an online patient information sheet, consent form and health questionnaire and invited to register to attend a clinic. Participants were eligible for the study if they were over 18 years of age. Exclusion criteria included anyone with a blood disorder or contraindication to giving a blood sample, or anyone currently exhibiting symptoms of COVID-19. To enrich the cohort for samples potentially positive for SARS-CoV-2 IgG antibody, further participants were invited if they had previously tested PCR positive or had the distinctive symptom of loss of taste and smell. Blood sampling clinics were held at locations around Northern Ireland between May and July 2020 resulting in

collection of 263 10ml EDTA plasma samples from 263 separate study participants. Additional anonymised plasma samples were obtained from Southern Health and Social Care Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood Transfusion Service (NIBTS, n=184) through convalescent plasma programs.

Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster University ethics committee approved studies with ongoing consent and from NIBTS (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-thaw cycles for all analyses reported within this manuscript.

Clinical information

Basic demographic information and data with regard to probable or definite prior infection with SARS-CoV-2 virus was obtained from PANDEMIC study participants through the secure online questionnaire requiring responses about positive RT-PCR result and/or time from symptom onset. Anonymised participant samples from USA, SHSCT and NIBTS were provided with age, gender and time since PCR-positive, where a previous test had been carried out.

Laboratory-based immunoassays

Details of laboratory immunoassays are summarised in supplementary methods and Table S1.

UK-RTC AbC-19 LFIA

UK-RTC AbC-19 testing was conducted at Ulster University according to manufacturer's instructions (details in Table S1). Assays were performed as cohorts,

with samples in batches of 10, with one researcher adding 2.5µL of plasma to the assay and a second adding 100µL of buffer immediately following sample addition. After 20 minutes, the strength of each resulting test line was scored from 0-10 according to a visual score card (scored by 3 researchers; Figure S2). A score ≥1 was positive. Details of samples used for analysis for detection of antibodies are available in Supplementary methods.

Statistical analysis

As per Daniel (9) a minimum sample size based on prevalence can be calculated using the following formula: $n = \frac{Z^2 P(1-P)}{d^2}$, where n = sample size, Z = Z statistic for a chosen level of confidence, P = estimated prevalence, and d = precision. Assuming a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the required sample size at 99% confidence (Z = 2.58) to be 240 individuals. If the true prevalence is lower, 5%, the estimated required sample size given a precision of 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody immunoassays(10).

Statistical analysis was conducted in in R v 4.0.2(11). To assess discordance between test results, data was first filtered to include individuals with an Abbott test result in the range ≥0.25 & ≤1.4, with a 2 x 2 contingency table produced that comprised all possible combinations of [concordant|discordant] test results [within|outside of] this range. A p-value was derived via a Pearson χ^2 test after 2000 p-value simulations via the stats package.

AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To

compare test result (Positive|Negative) to age, a binary logistic regression model was produced with test result as outcome – a p-value was then derived via χ^2 ANOVA. To compare time against test result (encoded continuously), a linear regression was performed. We calculated median per time-period and then converted these to log [base 2] ratios against the positivity cut-off for each assay. All plots were generated via ggplot2 or custom functions using base R(12).

Results

We analysed samples from a mixed cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations and research studies (n=223) and through a convalescent plasma program (n=183). Antibody levels in plasma from these 880 individuals were assessed using the three SARS-CoV-2 immunoassays; EuroImmun IgG, Roche Elecsys IgG/IgM/IgA and Abbott Architect IgG (Table S1). This included a cohort of 223 pre-pandemic plasma samples collected and stored during 2017 to end of May 2019 to determine assay specificity. Of the 657 participants whose samples were collected during the pandemic, 265 (40.33%) previously tested RT-PCR positive with a range of 7-173 days since diagnosis. A total of 225 participants gave time since self-reported COVID-19 symptoms, with a range of 5-233 days from symptom onset, whilst 198 had no symptom or PCR data available.

Laboratory based antibody immunoassays

A positive result for antibody on one or more of the three laboratory immunoassays was recorded for 385/657 (58.6%) participants who provided a sample during the

pandemic. By EuroImmun ELISA, 346 were positive, 20 borderline and 291 were negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott determined 310 positive and 347 negative (Table S2). The median age across all age groups combined was lower for participants testing positive across each of the immunoassays (median [sd] for positive versus negative, respectively: EuroImmun, 41 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41 [13.18] vs 47 [13.09]). (Figure S3, p<0.0001). When segregated by age group, however, differences were less apparent in certain groups (Figure S4). Excluding the pre-pandemic cohort, this gap reduced but remained statistically significant EuroImmun, 41 [13.18] vs 45 [12.49]; Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41 [13.26] vs 44 [12.63]) (p<0.01) (median [sd] for positive versus negative). Of note, out of 265 individuals with a previous positive RT-PCR result for SARS-CoV-2 viral RNA, 14 (5.2%) did not show detectable antibodies by any of the three immunoassays, with no association found with age, gender or time between test and blood draw (data not shown).

The three commercial laboratory immunoassays provide a ratio value that increases with IgG antibody titre. When correlation between these values is assessed, good overall agreement is observed between the three immunoassays (Figure 1, Figure S5). As highlighted by Rosadas *et al*., we also see significant disagreement in the Abbott 0.25-1.4 range when compared to EuroImmun and Roche (Figure 1a,b; chi-square p-values: EuroImmun vs Abbott, p<0.001; Roche vs Abbott, p<0.001)(13).

Duration of humoral response to SARS-CoV-2

We found IgG antibodies could still be detected in individuals (excluding pre-pandemic) across all three immunoassays used up to week 20 (day 140) (Figure 2). We note a statistically significant decrease in signal with respect to time across each

assay (p-value [slope]): EuroImmun, p=0.036 [-0.785]; Roche, p=0.002 [-0.125]; Abbott, p<0.0001 [-3.585]. These remained statistically significant after adjustment for age. Antibody levels (expressed as a ratio of median result per timepoint divided by positivity cut off; Table 2) peaked at Week 1-2 for EuroImmun (1.33) and Abbott (1.64), though reached highest levels at Week 8-12 when measured by Roche (5.45). By week 21-24, median score for all tests had dropped below the positivity cut off, though a small number of RT-PCR positive samples remained above the positive cut off at these later timepoints (Figure 2).

UK-RTC AbC-19

Using the commercial immunoassays described we established a well characterised serology sample set of 'known positive' and 'known negative' for IgG antibodies to SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.

AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore required all samples to be positive by the EuroImmun SARS-CoV-2 IgG ELISA, which likewise detects antibodies against the S1 domain (14). To develop this characterised cohort, samples were also required to be positive by a second immunoassay (Roche or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG antibody, we assessed 350 plasma samples from participants classed as 'known negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from individuals confirmed to be negative across all three laboratory assays (Roche, EuroImmun, Abbott). Using these positive n=304 and negative n=350 antibody cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the AbC-19 LFIA (Table 1).

Given a recent report of lower specificity in the AbC-19 LFIA (15) and the possibility of introducing sample bias, we revised our inclusion criteria for the negative cohort. For the pre-pandemic cohort, we included samples from all 223 individuals, regardless of results on other laboratory immunoassays. When this assumed negative pre-pandemic cohort was used for laboratory evaluation for target condition of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 1). We obtained more AbC-19 devices and expanded the negative cohort to include all samples that matched our criteria (samples collected during the pandemic to be negative by all three laboratory assays and all pre-pandemic samples regardless of other immunoassay results). The specificity observed on this extended negative cohort of 488 samples was 99.59% (98.53% to 99.95%, Table 1). For sensitivity analysis on a positive cohort (samples positive by EuroImmun and one other test), we were able to analyse all samples previously untested due to limited testing capacity and tested a positive cohort of 330 samples giving a sensitivity of 97.58% (95.28% to 98.95%, Table 1).

When used for its intended use case, the AbC-19 LFIA provides binary positive/negative results. However, when assessing LFIA in the laboratory, each test line was scored against a scorecard by three independent researchers (0 negative, 1-10 positive; Figure S2). Compared to quantitative outputs from the Abbott, EuroImmun and Roche assays, the AbC-19 LFIA shows good correlation (Abbott r=0.84 [p<0.001]; EuroImmun r=0.86 [p<0.001]; Roche r=0.82 [p<0.001]; Figure 3, Figure S5-Figure S7).

Analytical specificity and sensitivity of AbC-19 LFIA

We observed no cross-reactivity across samples with known H5N1 influenza, Respiratory syncytial virus, Influenza A, Influenza B, Bordetella Pertussis, Haemophilus Influenzae, Seasonal coronavirus NL63 and 229E on the AbC-19 LFIA (n=34 samples, n=8 distinct respiratory viruses; Table S3). Against a panel of external reference SARS-CoV-2 serology samples, the AbC-19 LFIA detected antibodies with scores commensurate to the EuroImmun ELISA scores (Figure S8, Table S4).

Discussion

Serological antibody immunoassays are an important tool in helping combat the SARS-CoV-2 pandemic. One difficulty faced in validation of antibody diagnostic assays has been access to samples with known SARS-CoV-2 antibody status. As previously described, there is no clear gold standard for reference against which to assess SARS-CoV-2 immunoassays. A positive RT-PCR test has been used previously to indicate previous COVID-19 infection, though this approach is limited by a high rate of false negatives, failure in some cases to develop IgG antibodies (sero-silence or lack of antibody against the same antigenic component of the virus as the immunoassay uses as a capture antigen) and the lack of RT-PCR testing availability early in the pandemic (3,5,16). We failed to detect SARS-CoV-2 IgG antibody in 14 of 265 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA positive participants in this study. It is unclear if this is due to insufficient/absent antibody production in these individuals, or due to a false positive PCR result which may occur in the UK at a rate between 0.8- 4.0% (17). Self-assessment of symptoms for COVID-19 disease is a poor indicator of previous infection, even amongst healthcare workers (18). Asymptomatic individuals may be unaware of infection and others may harbour pre-existing immunity or elucidate a T cell response. Additionally, the kinetics of a SARS-

CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus with time, contributing to false negative RT-PCR test results for individuals who may be late to present for virus detection tests (5,19).

Our results show strong correlation between all three immunoassays, with shortcomings in the Abbott system output 0.25-1.4 range, as described previously, suggesting an overestimated positive cut-off (Figure 1) (13). Our detection of antibodies 140 days after RT PCR positive status (20 weeks, and beyond in a small number of samples) indicates persistence IgG antibodies to both the spike protein and nucleocapsid protein, despite typical patterns of antibody decay after acute viral antigenic exposure being as rapid (20). Others have reported SARS-CoV-2 antibodies decline at 90 days (19), we also noted a statistically significant decline over time but levels remain detectable at 140 days (Figure 2). We note that IgG levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as Week 8-12 from first symptoms or a viral RNA RT-PCR positive result, though this may be an artefact of lower number of participants at earlier timepoints (Table 2). Longitudinal studies on SARS-CoV-1 convalescent patients suggests that detectable IgG can still be present as long as 2 years after infection (21). Further studies are needed on large cohorts with sequential antibody immunoassays performed on symptomatic and non-symptomatic individuals as well as those with mild or severe COVID-19 to fully elucidate the humoral immune response to SARS-CoV-2. This is vital to inform vaccine durability, so-called ‘immune passports’ and in the definition of a protective threshold for anti-SARS-CoV-2 antibodies.

To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-CoV-2 antibody in a laboratory evaluation, we developed a reference standard for SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar approach was used in a recent seroprevalence study in Iceland, whereby two positive antibody results were required to determine a participant sample as positive for SARS-CoV-2 antibody (16).

Our evaluation of performance metrics for the UK-RTC AbC-19 LFIA to detect antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59% specificity. In a recent evaluation of the AbC-19 tests, Mulchandani et al. observed a specificity of 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report a sensitivity of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a previous RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys antibody test, which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid portion of SARS-CoV-2 (18).

In our study, good correlation was observed in quantitative score between results on all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA (Figure S6, S7). This is to be expected, given both the AbC-19 LFIA and EuroImmun ELISA detect IgG antibodies against spike protein. For the assessment of immunity to prior natural infection as well as to immunisation, it is important to note IgG antibodies against SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA and AbC-19 LFIA are known to correlate with neutralizing antibodies, which may confer future immunity (22,23).

Previous evaluations of the sensitivity and specificity reported by Public Health England (PHE), showed a EuroImmun sensitivity of 72% and specificity of 99%, Abbott with sensitivity of 92.7% and specificity of 100% and Roche with sensitivity of 83.9% and specificity of 100% (24–26). The PHE analyses for each of these tests used previous infection (RT-PCR positive status) as a reference standard, the limitations of which are discussed above.

In the use of characterised 'known positive' and 'known negative' cohorts, one limitation of this study is its potential for spectrum bias, whereby our positive-by-two reference system may artificially raise the threshold for positive sample inclusion, possibly resulting in the overestimation of the sensitivity of any test evaluated (27). However, similar issues have been raised when using previous RT-PCR result or definitive COVID-19 symptoms as inclusion criteria given these will likely skew a cohort towards more severe disease (5). Importantly, our mixed origin of samples forming the cohort provides a positive cohort for assessing assay sensitivity that includes individuals from the general public, healthcare workers and from convalescent plasma programmes. Our analysis of specificity on only pre-pandemic individuals (n=223) shows similar specificity (99.55%) to the larger mixed 'known negative cohort' (n=488, sensitivity 99.59%). In the absence of a clear gold standard test, our system relies on no single test (each with their individual shortcomings) and instead takes an average of three.

Our assessment of the UK RTC AbC-19 LFIA using our characterised cohorts of known SARS-CoV-2 antibody positive and antibody negative plasma, in a laboratory setting shows good performance metrics for its ability to detect SARS-CoV-2 IgG

antibody. We note it uses plasma from venous blood samples, as opposed to the use of a finger prick blood sample. Additionally, when this UK RTC AbC-19 LFIA was used on our cohort, a number of the positive results scored low, (1/10 using the score card under laboratory conditions, Figure 3) with a faint test band visible to a trained laboratory scientist but perhaps difficult to identify as positive by individuals performing a single test (Figure S6). This faint line may be reflective of the longer time from infection for the Northern Ireland cohort used. If this AbC-19 LFIA is to be used in clinical settings it is important to determine if all users observe the same results as observed in this laboratory evaluation.

This assessment of the AbC-19 LFIA does not provide data on how this test will perform in a seroprevalence screening scenario, but instead provides metrics for the performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as opposed to previous COVID-19 infection. An important potential use of the AbC-19 LFIA would be in monitoring the immune response to vaccination, with most vaccines utilising SARS-CoV-2 Spike protein antigens (28). It is not yet known if presence of SARS-CoV-2 antibodies indications immunity from infection.

Conclusion

We present a comprehensive analysis of 880 pre-pandemic and pandemic individuals and show IgG antibodies are detectable up to 140 days from symptoms or positive RT-PCR test, showing persistence of immunity at later time points than previously published. We use antibody positive as an alternative to RT-PCR positive status as a standard for assessing SARS-CoV-2 antibody assays and show strong performance for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-CoV-2

antibodies. It is fully understood that user experience in future studies in the real world is important and may alter the performance characteristics. Also, the effect of operator training will have direct effects upon test performance. We welcome further clinical evaluation of the AbC-19 LFIA in large cohorts of symptomatic and asymptomatic individuals alongside large studies assessing COVID-19 outcomes in individuals with longitudinal studies to fully validate its implementation across all intended use cases.

Declarations

Ethics approval and consent to participate

All study participants provided informed consent. This study was approved by Ulster University Institutional Ethics committee (REC/20/0043), South Birmingham REC (The PANDEMIC Study IRAS Project ID: 286041Ref 20/WM/0184) and adhered to the Declaration of Helsinki and Good Clinical Practice.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Consent for publication

Not applicable.

Dissemination to participants and related patient and public communities.

Links to this work will be included on the study website (https://www.ulster.ac.uk/coronavirus/research/research-output/pandemic-study) and participants will be alerted that the work has been published.

Data sharing

Data are available on reasonable request to the corresponding author.

Competing interests:

At the time of this study TM and JML acted as advisors to CIGA HealthCare, an industrial partner in the UK Rapid Test Consortium. No personal financial reward or renumeration was received for this advisory role. At the time of submission of this manuscript TM and JML no longer held these advisory positions.

All other authors have no potential conflict of interest to report.

Funding:

Costs for assays and laboratory expenses only will be paid by UK-RTC as is normal practice (UU-UK-RTC-2020-001). The authors have not been paid or financially benefitted from this study.

The advisory roles within CIGA Healthcare were unpaid temporary roles. This manuscript and associated data within this paper has only been used to build confidence into the overall device design and performance assessment of the UK RTC AbC-19 devices and such work was never commissioned for any government contractual consideration.

Authors' contributions:

TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR, SM and KYN analysed data, KB performed all statistical analyses/interpretations and produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and provided Blood Transfusion cohort samples. TM, RP and AN coordinated participant recruitment, consent and sampling. WB and JML developed online consent forms, questionnaires and databases. LR, JM, AK, AA, GW, DH, SS, CCS performed sample collection and processing. LR and TM wrote the manuscript, with significant contributions from JM and KB. All authors reviewed and approved the final manuscript.

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Table 1: UK-RTC AbC-19 LFIA performance metrics against known antibody positive and known antibody negative cohorts.

Total Negative	True Negative	False Positive	Total Positive	True Positive	False Negative	Sensitivity % (95 CI)	Specificity % (95 CI)
Pre-pandemic (n=223)							
223	222	1	n/a	n/a	n/a	n/a	99.55% (97.53% to 99.99%)
Initially reported cohorts (n=654)							
350	350	0	304	297	7	97.70% (95.31%-99.07%)	100.00% (98.95%-100.00%)
Extended cohorts (n=818)							
488	486	2	330	322	8	97.58% (95.28%-98.95%)	99.59% (98.53%-99.95%)

Table 2: Antibody level ratios for assays over time

	Ratio Antibody level:assay positivity cut-off									
	Week									
	Pre-2020	1-2	3-4	5-8	9-12	13-16	18-20	21-24	25-28	29+
EuroImmun	-2.65	1.33	0.2	0.87	1.32	0.47	0.04	-2.01	-2.26	-2.01
Roche	-3.64	3.16	3.05	5.21	5.45	4.14	4.42	-3.54	-3.69	-3.61
Abbott	-5.54	1.64	-0.51	0.99	0.86	0.08	-0.59	-5.13	-5.13	-6.13
Sample number (n=)	223	20	10	50	90	202	53	11	12	11

Antibody level ratios for assays over time show varying peaks levels depending on test. Calculated by first establishing the median per time period, then calculating log2 ratio for each period versus each respective assay positivity cut-off.

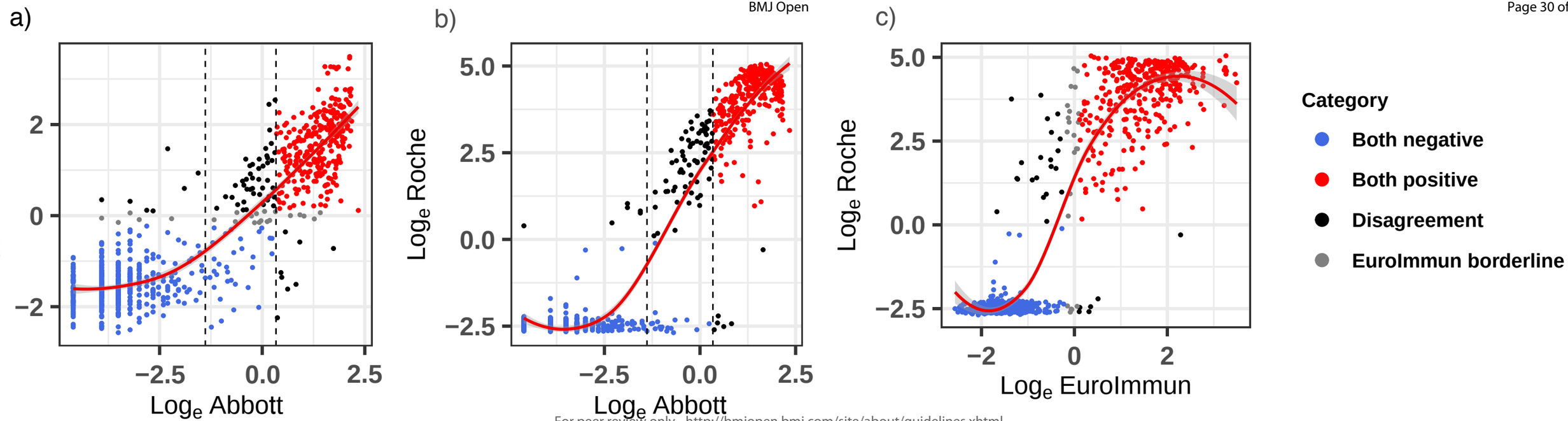
Figure Legends

Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott and c) Roche immunoassays. Pearson χ^2 test was used to assess correlations. The results for each test were log transformed to ensure results follow a normal distribution. Negative agreement shown as blue dots, red dots show positive agreement for the two immunoassays, whilst black dots show disagreement and grey dots as the EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4. n=880. The graphs show positive correlations between all immunoassays evaluated, with the fewest disagreement of results between the Log of Roche and the Log of EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.

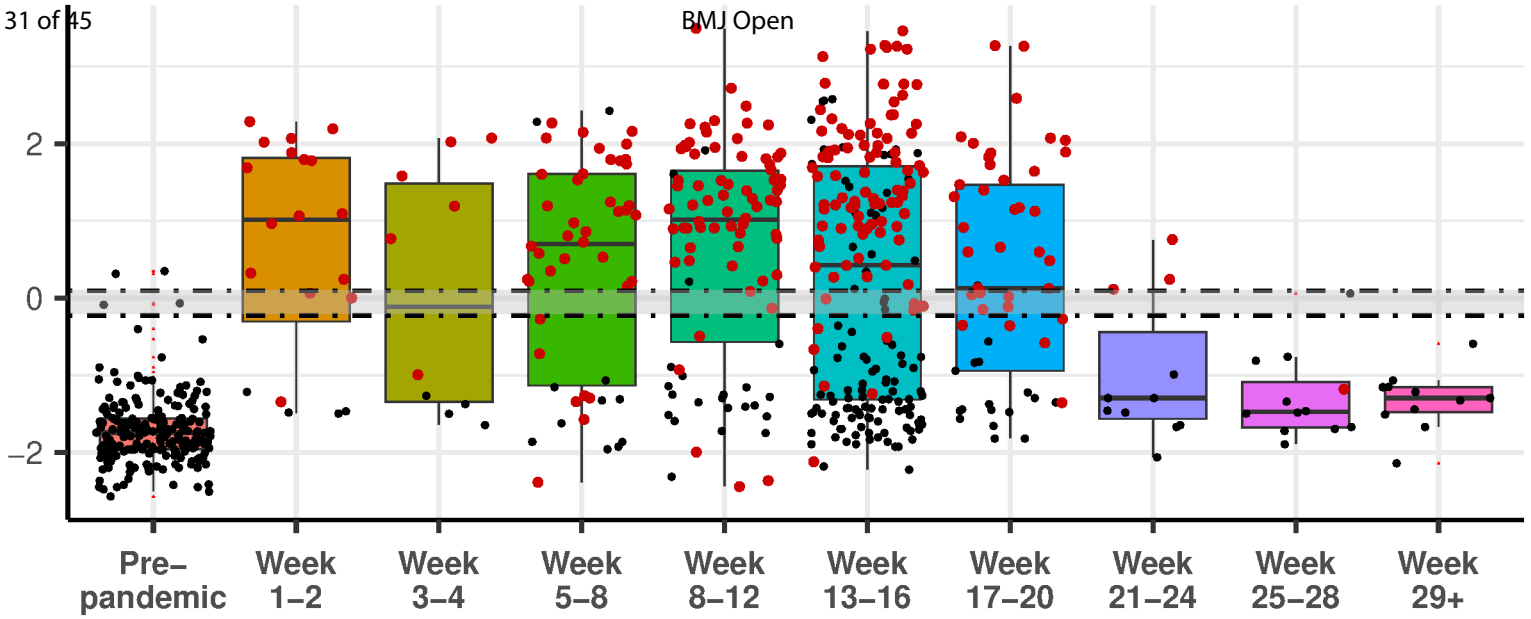
Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c) Abbott, relative to weeks since first reported symptoms or positive PCR result (where data available, n=682). RT-PCR positive individuals are denoted by red dots, while individuals with time since symptom data are denoted in black. Dashed lines delineate $\log_e$ equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result between the two lines). Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).

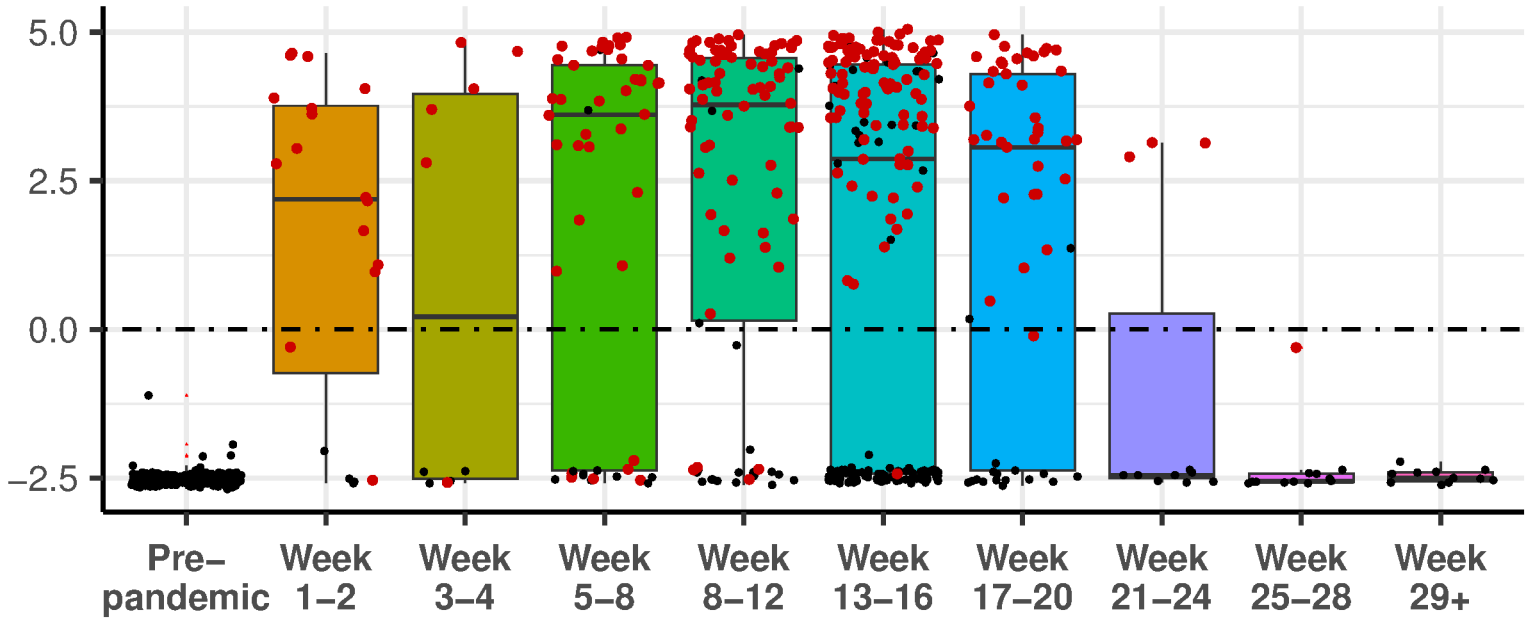
Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing AbC-19 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line

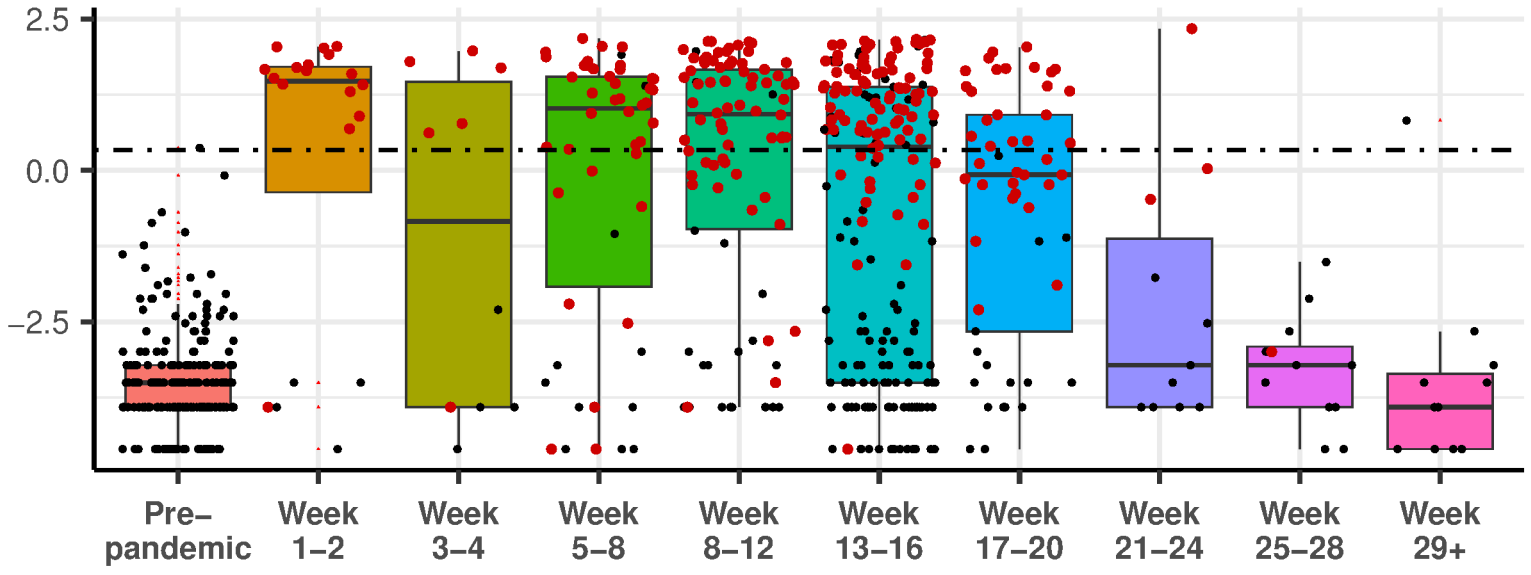
of best fit with 95% confidence interval shaded in grey. Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).

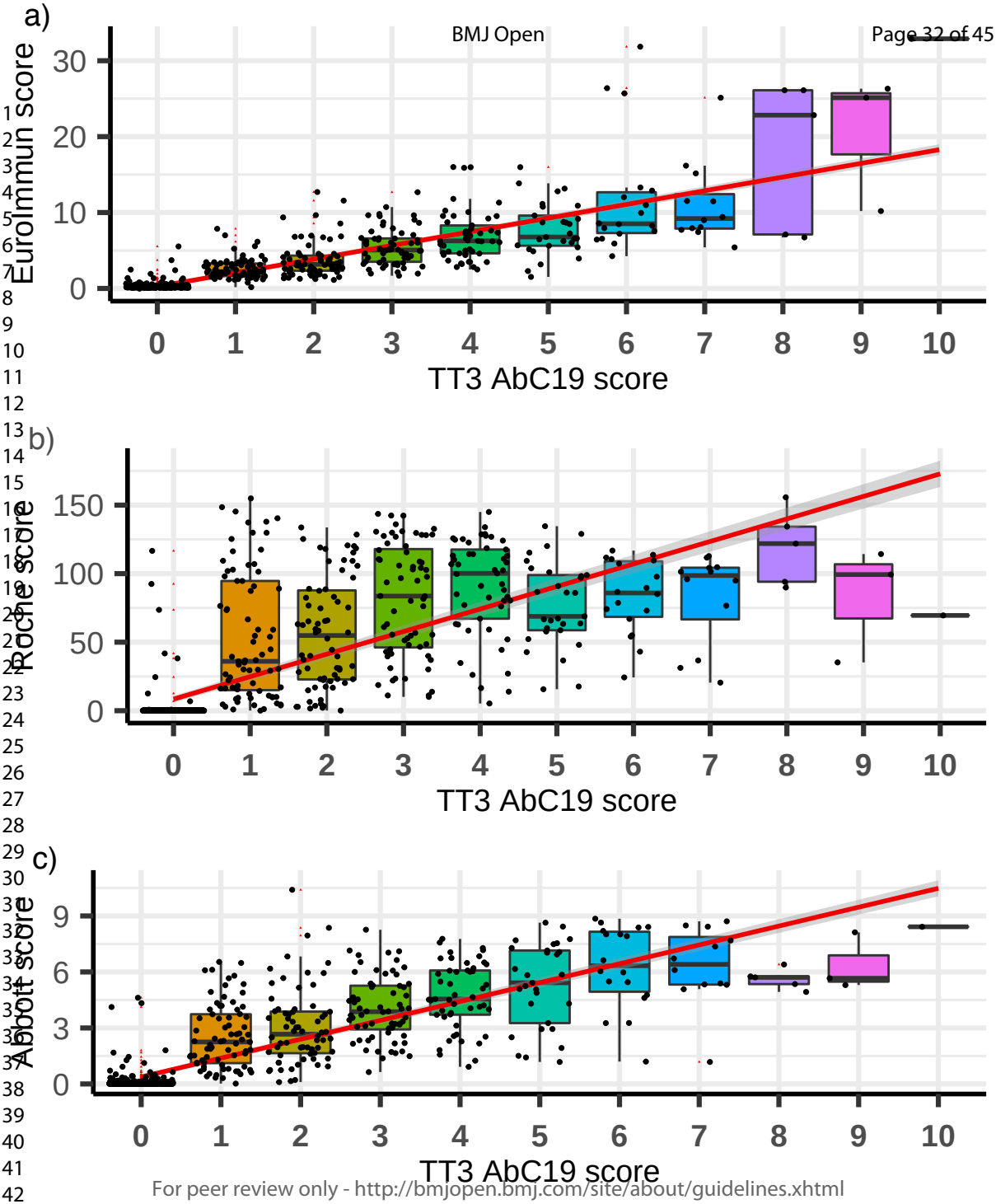


Fit lines, LOESS with 95% CI
Vertical lines mark Abbott test range 0.25−1.4


a)
Log$_e$ EuroImmun
2
0
−2
Pre–pandemic
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+


b)
Log$_e$ Roche
5.0
2.5
0.0
−2.5
Pre–pandemic
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+


c)
Log$_e$ Abbott
2.5
0.0
−2.5
Pre–pandemic
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+



Linear fit line with 95% CI

Supplementary Materials

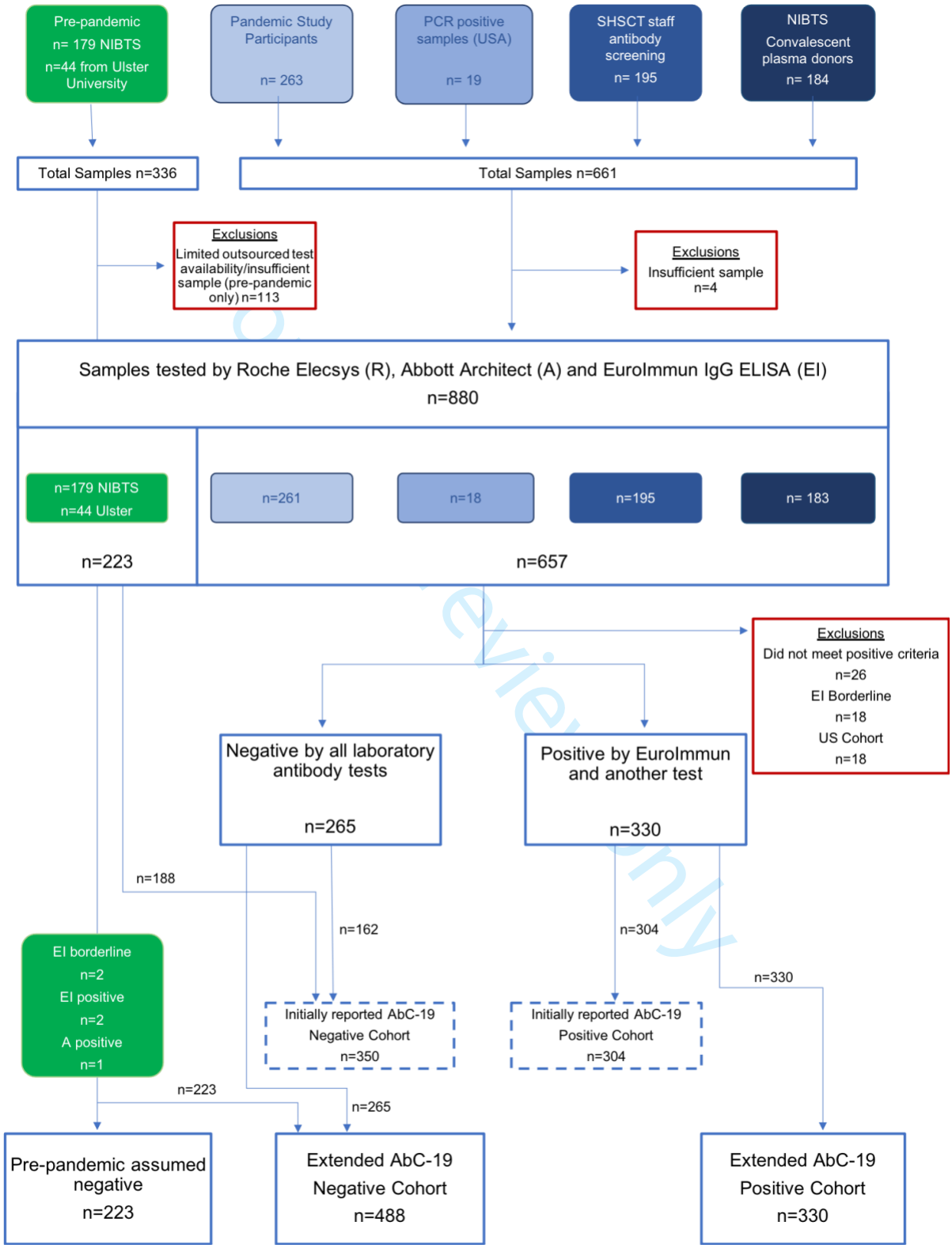


Figure S1: Flow of participant plasma samples through the study.

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

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

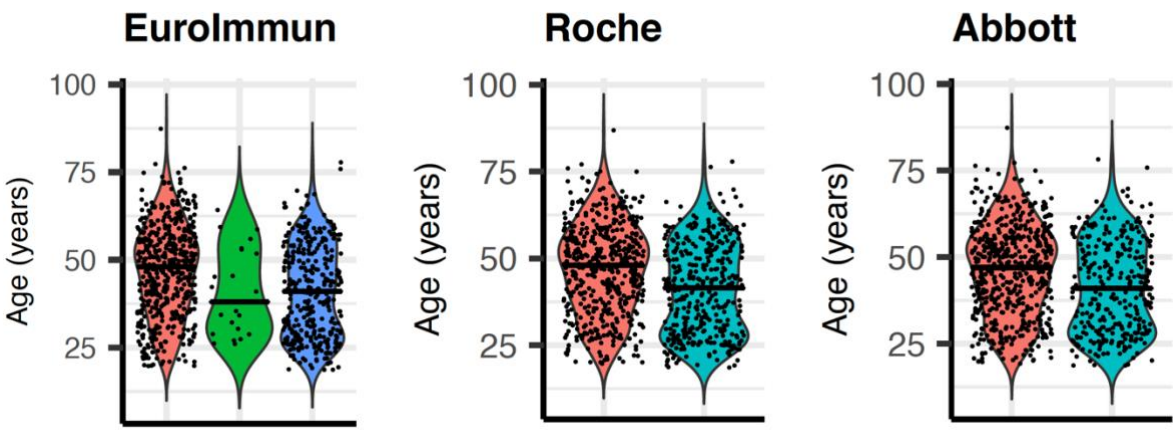


Figure S3: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.

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

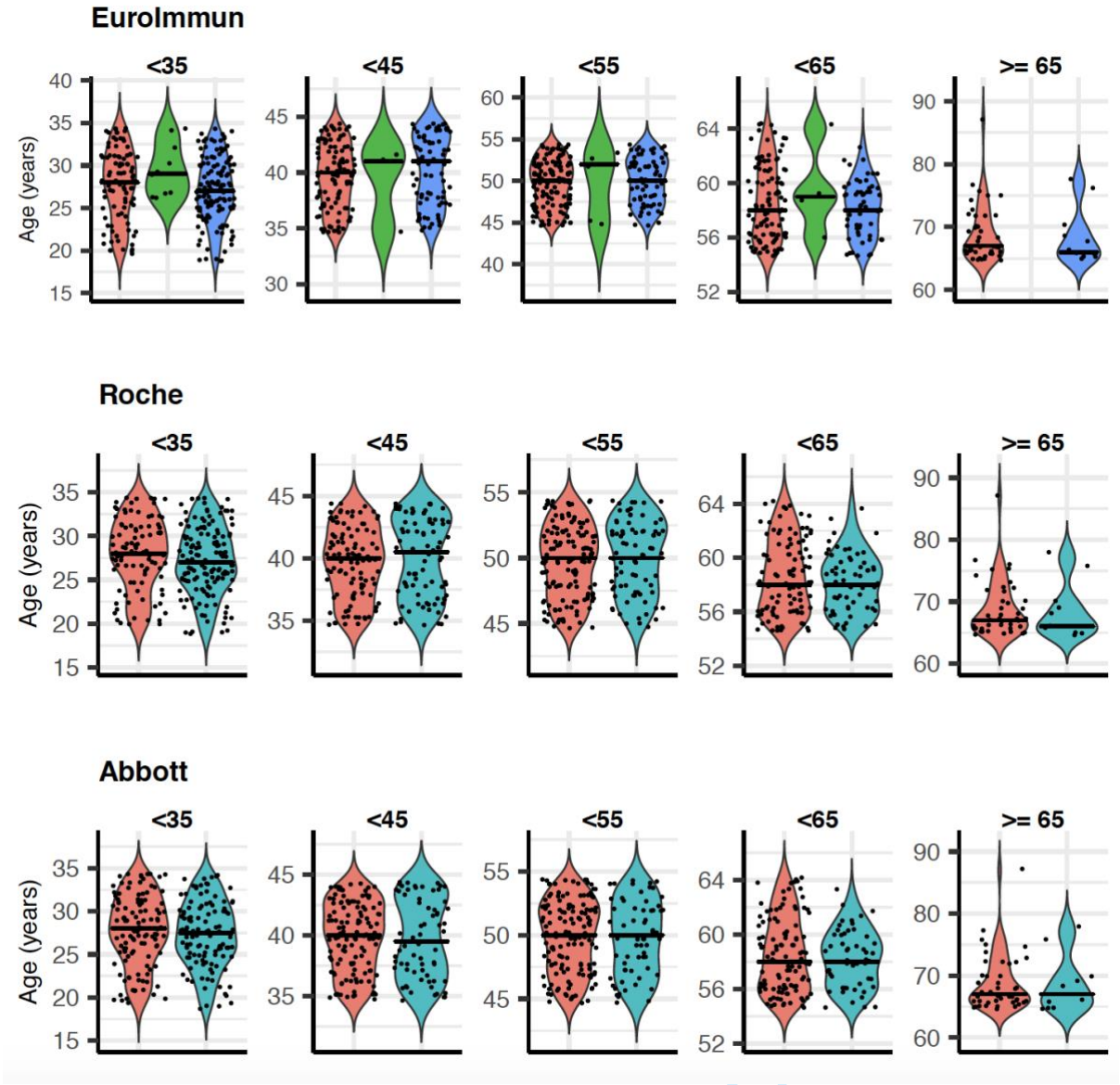


Figure S4: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.

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

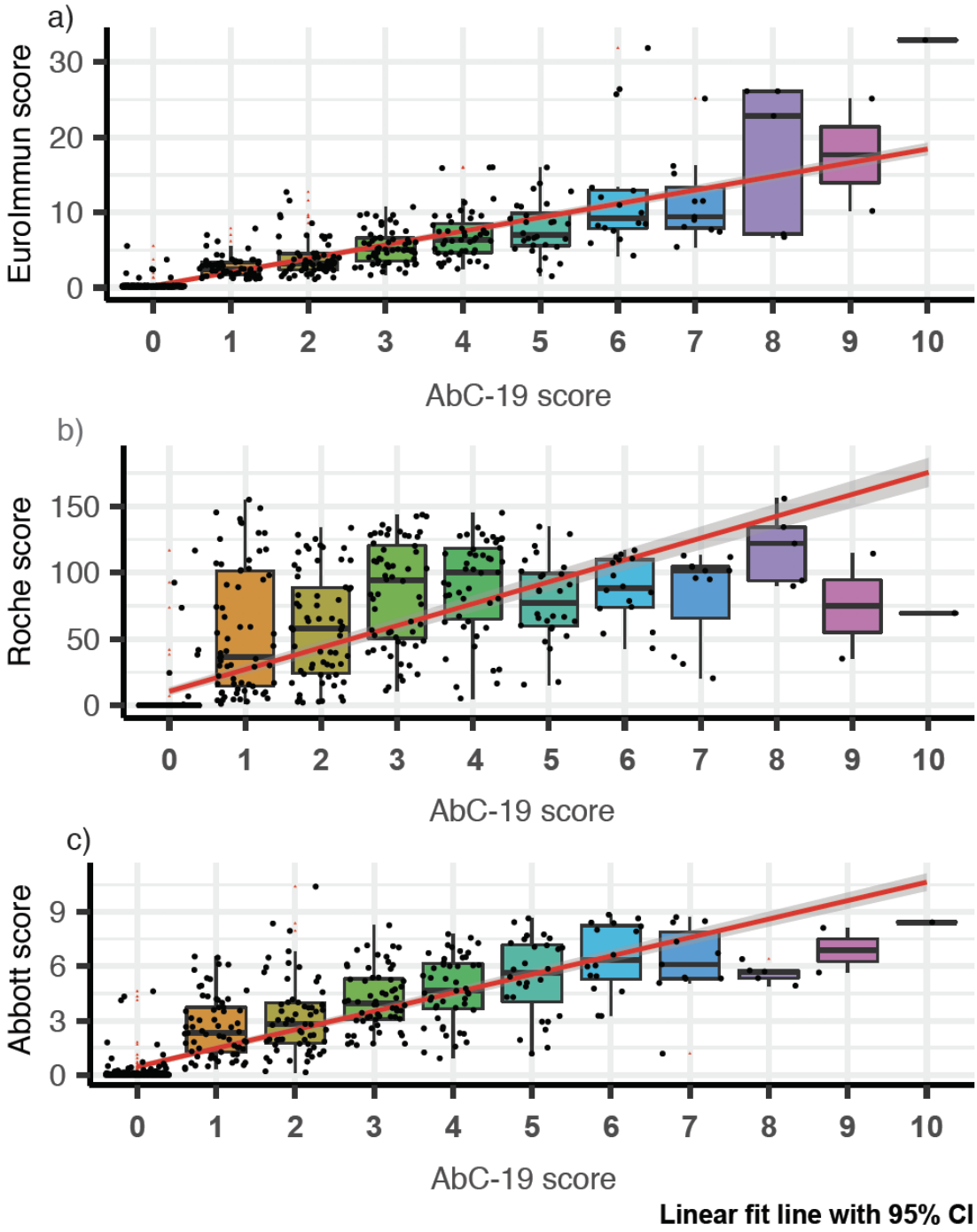


Figure S5: AbC-19 initially reported cohort n=654 correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing AbC-19 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line of best fit with 95% confidence interval shaded in grey. Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).

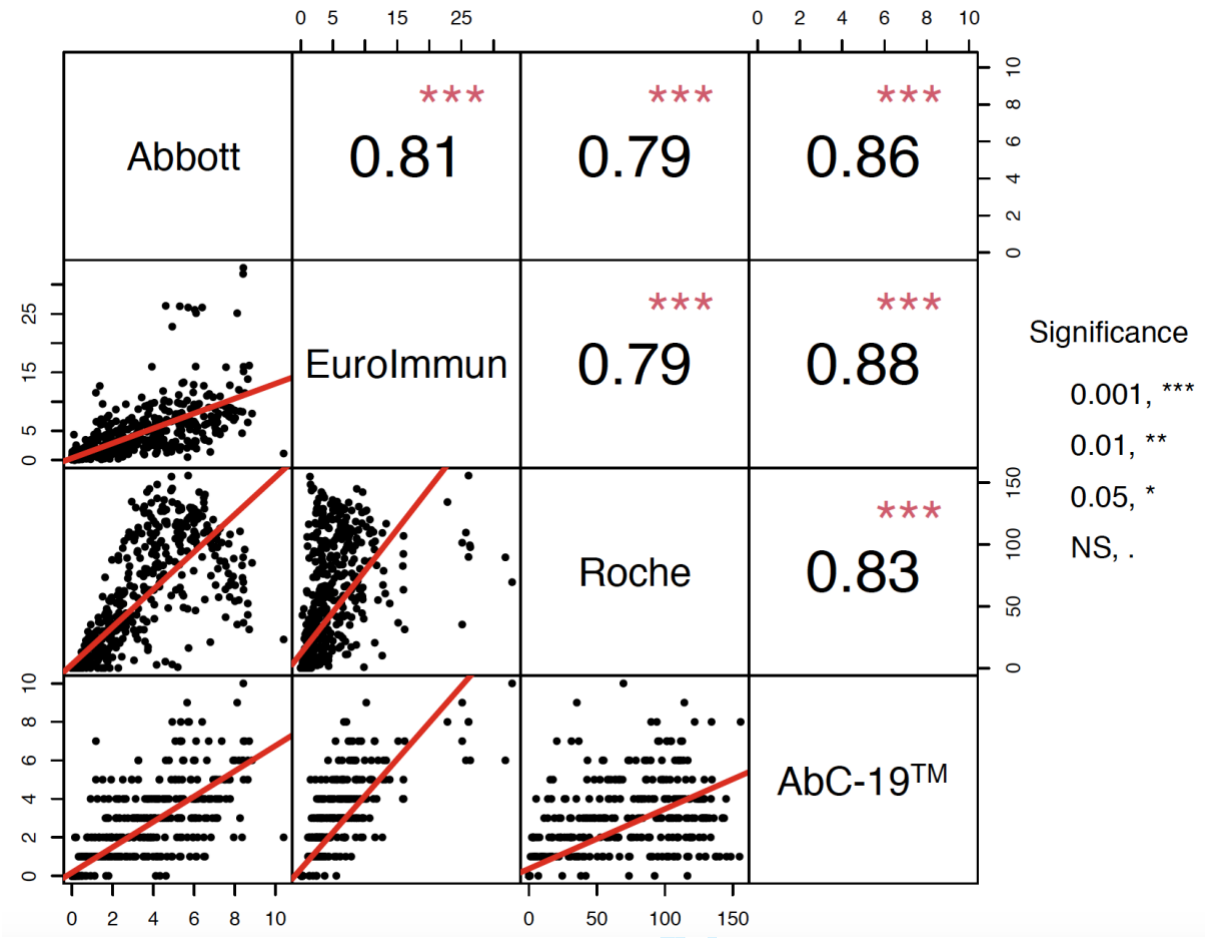


Figure S6: **Correlation matrix between Abbott, EuroImmun, Roche and initially reported AbC-19 cohort (n=654) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly correlated $p<0.001$.

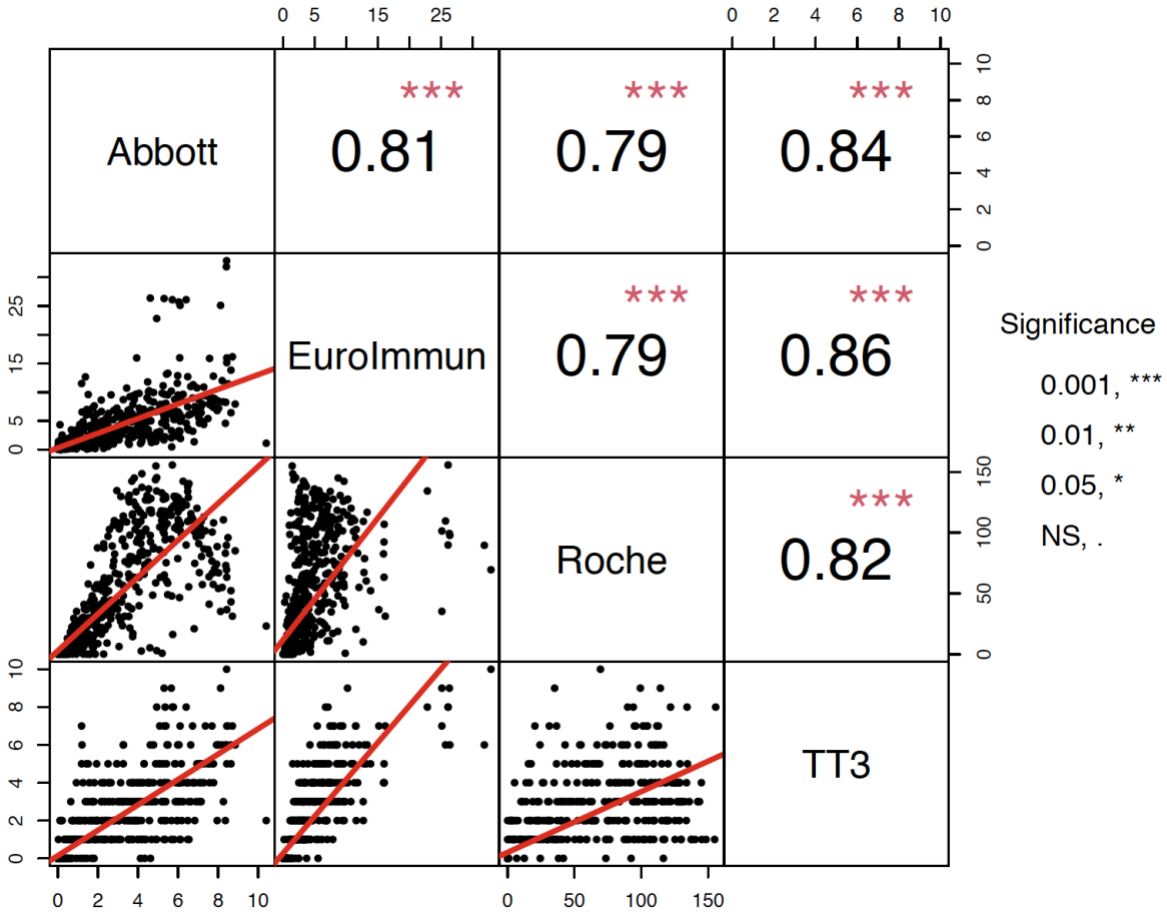


Figure S7: **Correlation matrix between Abbott, EuroImmun, Roche and extended AbC-19 cohort (n=818) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly correlated $p<0.001$.

Figure S8: NIBSC external reference serology standards and known respiratory virus serology samples.

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

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

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

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

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

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

Table S3: Analytical specificity analysis on the AbC-19 LFIA LFIAs were assessed using 34 serum samples with known other respiratory viruses, negative results for all suggests analytical specificity for SARS_CoV_2 IgG.

SAMPLE	Number of samples	Number of AbC-19 Positive results	Number of AbC-19 Negative results
H5N1 Influenza (NIBSC 7/150)	1	0	1
RSV (NIBSC 16/284)	1	0	1
Influenza B (NIBSC 9/222)	1	0	1
Bordetella Pertussis (NIBSC 89/530)	1	0	1
Influenza A	5	0	5
Influenza B	5	0	5
Respiratory syncytial virus	5	0	5
Haemophilus Influenzae	5	0	5
Seasonal coronavirus NL63	5	0	5
Seasonal coronavirus 229E	5	0	5

Table S4: AbC-19 LFIA results with NIBSC external reference samples

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

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

Supplementary Methods

Laboratory-based immunoassays

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

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

determined negative, ≥ 0.8 to <1.1 borderline and ≥ 1.1 positive. For a portion of samples provided by NIBTS, EuroImmun IgG assay data was provided to researchers by NIBTS.

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

Abbott Architect SARS-CoV-2 immunoassay was carried out according to manufacturer's instructions on the Abbott Architect i2000SR analyser (Abbott, kit 18115FN00, calibrator kit 17412FN00, Control kit 17531FN00). The external control is entered into a Quality Monitor programme and must be within 3 standard deviations of the mean (cumulative; External control NIBSC QCRSARSCoV-2QC1 Lot 20/B764-01). Results are reported by dividing the sample result by the calibrator result. The result unit for the SARS-CoV-2 IgG assay is Index (Sample/Calibrator). A ratio of < 1.4 is determined negative and ≥ 1.4 is determined positive.

Analytical specificity and sensitivity assessment

Four virology samples (H5N1 influenza serology 7/150, RSV serology 16/284, Influenza B 9/222 and Bordetella Pertusis 89/530) were obtained from NIBSC (National Institute for Biological Standards, Herts, UK). An additional 30 serology

samples from known virus infections were a kind gift from SugenTech, Soeul, Korea. 15 of these virology samples were obtained from Trina (Trina Bioreactives AG, Switzerland) from 5 different individuals per virus (Influenza A IgG, Influenza B IgG and RSV IgG). A further 15 of these virology samples were obtained from AbBaltris, Kent from 5 different individuals per virus (Haemophilus Influenza IgG, Seasonal Coronavirus NL63 and 229E Seasonal Coronavirus). All these serology samples alongside a panel of 6 external standard research reagents (Table S4; NIBSC; Cat: 20/118 and 20/130) were assessed on the AbC-19 LFIA to confirm analytical specificity and sensitivity.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	3-4
	4	Study objectives and hypotheses	4-5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**6**	Eligibility criteria	5
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5/6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5/6
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	7
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	4
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	7, supp table 1
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Supp methods, supp table 1
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Supp methods
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	7/8
	15	How indeterminate index test or reference standard results were handled	10, Supp Fig1
	16	How missing data on the index test and reference standard were handled	Supp Fig 1
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	11
	18	Intended sample size and how it was determined	7
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Supp Fig 1
	20	Baseline demographic and clinical characteristics of participants	8/9
	21a	Distribution of severity of disease in those with the target condition	9
	21b	Distribution of alternative diagnoses in those without the target condition	9
	22	Time interval and any clinical interventions between index test and reference standard	5
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Fig 3, Supp Fig 5-7
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10/11, Table 1
	25	Any adverse events from performing the index test or the reference standard	5
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	14/15
	27	Implications for practice, including the intended use and clinical role of the index test	15
OTHER INFORMATION			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents

	30	Sources of funding and other support; role of funders	18

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Laboratory evaluation of SARS-CoV-2 antibodies: detectable IgG more than 10 months post infection.

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Abstract

Objective

To evaluate the dynamics and longevity of the humoral immune response to SARS-CoV-2 infection and assess the performance of professional use of the UK-RTC AbC-19 Rapid Test lateral flow immunoassay (LFIA) for the target condition of SARS-CoV-2 spike protein IgG antibodies.

Design

Nationwide serological study.

Setting

Northern Ireland, UK, May 2020- February 2021.

Participants

Plasma samples were collected from a diverse cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations and research studies (n=223) and through a convalescent plasma program (n=183). Plasma donors (n=101) were followed with sequential samples over 11 months post symptom onset.

Main Outcome Measures

SARS-CoV-2 antibody levels in plasma samples using Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2 ELISA immunoassays over time. UK-RTC AbC-19 LFIA sensitivity and specificity, estimated using a three-reference standard system to establish a characterised panel of 330 positive and 488 negative SARS-CoV-2 IgG samples.

Results

We detected persistence of SARS-CoV-2 IgG antibodies for up to 10 months post infection, across a minimum of two laboratory immunoassays. On the known positive cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58% (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-99.95%).

Conclusions

Through comprehensive analysis of a cohort of pre-pandemic and pandemic individuals, we show detectable levels of IgG antibodies, lasting over 46 weeks when assessed by EuroImmun ELISA, providing insight to antibody levels at later time points post-infection. We show good laboratory validation performance metrics for the AbC-

19 rapid test for SARS-CoV-2 spike protein IgG antibody detection in a laboratory-based setting.

Strengths and Limitations

Strength - This paper describes a non-clinical laboratory evaluation and comparison of the ability of three different immunoassays to detect SARS-CoV-2 antibodies in the same samples, detecting different subtypes of antibodies against different targets of the viral antigenic repertoire, that does not rely on PCR-positivity as definition of expected test outcome, to provide a panel of known antibody positive and antibody negative serology for evaluation of newly developed immunoassays.

Strength - This study demonstrates AbC-19 lateral flow point of care detection of IgG antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the antibodies made in response to the vaccines used globally, in a large cohort of subjects, more than 10 months post infection, across a broad age range (18-78 years). Robust antibody responses were observed in all age groups tested, including over-65s, who are most at risk of severe COVID-19 symptoms, and were prioritised in the UK-wide mass vaccination programme.

Strength - This study shows excellent correlation between approved laboratory-based assays and the AbC-19 lateral flow point of care lateral flow test for the detection of SARS-CoV-2 antibodies in characterised cohorts of known positive and negative plasma samples in an evaluation conducted according to MHRA guidelines during a pandemic.

Strength- Longitudinal data detecting IgG antibodies more than 10 months from infection was collected as sequential samples over time through a convalescent plasma donation program.

Limitation- This study was conducted in a standardised setting with very experienced users on plasma characterised as positive or negative for the presence of antibodies using a reference standard, alongside one other assay which may introduce a possible spectrum bias. The laboratory setting may not reflect the true performance metrics of the assay evaluated when translated to real life settings, using finger prick blood samples and in which pre-test probability would impact greatly on positive and negative predictive values.

Keywords

SARS-CoV-2, COVID-19, immunoassay, LFIA, pandemic, antibody assay

Introduction

The World Health Organization declared a pandemic in March 2020 due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), identified late 2019 in Wuhan, China, causing Coronavirus Disease 2019 (COVID-19) disease (1,2).

A global race ensued to develop diagnostic assays, with the most common being viral RNA detection (RT-qPCR assays), to detect acute infection(3). RT-qPCR assays are labour and reagent intensive, limited by a short temporal window for positive diagnosis, and exhibit potential for false negative results (4). Evidence suggests sensitivity of RT-qPCR can be as low as 70% (5). False positive rates between 0.8- 4.0% have been reported in the UK and are dependent on the Ct values accepted as indicating

infection, the number of SARS-CoV-2 genes analysed, and the proportion of asymptomatic individuals tested (6,7). Lockdown measures and "flattening the curve" strategies in the UK meant many infected individuals were instructed to self-isolate and were not offered a diagnostic RT-qPCR, with much of the testing limited to patients admitted to hospital, who perhaps reflect a more severely infected cohort. Consequently, a potentially large number of cases were unconfirmed or undetected (8).

The ability to accurately detect SARS-CoV-2 specific antibodies, which develop after an immune response is evoked, is vital for building biobanks of convalescent sera for treatment, monitoring immune response to infection alongside surveillance studies and assessing responses to vaccination programmes.

Commercial serology immunoassays are mostly laboratory-based and measure IgG antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIAs), require a finger prick blood sample and can be used at point-of-care (POC) or in the home; particularly important in the context of lockdown enforcement during the pandemic. A limited number of laboratory-based chemiluminescence immunoassays are approved for use in the UK including the Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region (Roche Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay against the same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

The complexities of the humoral immune response to SARS-CoV-2 is a much-debated topic. In a US study, approximately one in 16 individuals lacked detectable IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR confirmed infection (9). Patients who remain asymptomatic may mount a humoral immune response which is short-lived, with detectable levels of antibody falling

rapidly (10). This, alongside the lack of RT-PCR test availability across the UK has hindered development of well characterised gold standard serology test for IgG antibodies to SARS-CoV-2.

Herein, we describe the use of Roche and Abbott commercial immunoassays, as well as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike (antigenic) protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-pandemic and pandemic COVID-19 blood samples (n=880) from within Northern Ireland and report on longevity of IgG antibodies detected. Furthermore, we follow IgG antibody levels in convalescent plasma donors (n=101 individuals) for up to 11 months. Currently, there is no gold standard assay for comparison, therefore we aimed to establish a reference based on a positive COVID-19 antibody status. We present results of a laboratory evaluation of the UK-RTC AbC-19 with a target condition of antibodies against a cohort of 330 known IgG antibody positive samples according to this 'positive by two' system and 488 negative samples (223 pre-pandemic assumed negative and 265 known negative) for IgG to SARS-CoV-2.

Methods

Participant samples

The flow of participant samples is summarised in Figure S1. A small cohort (n=19) of anonymised plasma samples were obtained from a partner USA laboratory for initial protocol development only. All participants provided informed consent. An online recruitment strategy was employed, with the study advertised through internal Ulster University email, website and social media. A BBC Newsline feature providing the pandemic study email address also prompted interest from the general population.

The first 800 respondents who expressed interest were provided with an online patient information sheet, consent form and health questionnaire and invited to register to attend a clinic. Participants were eligible for the study if they were over 18 years of age. Exclusion criteria included anyone with a blood disorder or contraindication to giving a blood sample, or anyone currently exhibiting symptoms of COVID-19. To enrich the cohort for samples potentially positive for SARS-CoV-2 IgG antibody, further participants were invited if they had previously tested PCR positive or had the distinctive symptom of loss of taste and smell. Blood sampling clinics were held at locations around Northern Ireland between May and July 2020 resulting in collection of 263 10ml EDTA plasma samples from 263 separate study participants. Additional anonymised plasma samples were obtained from Southern Health and Social Care Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood Transfusion Service (NIBTS, n=184) through convalescent plasma programs. NIBTS convalescent plasma samples continued to be collected throughout 2020-early 2021, with a total of n=897 from n=676 individuals, including n=183 samples from the cross-sectional cohort. Individuals from this program with a positive RT-PCR result and EuroImmun starting value >6 were sequentially sampled over a period of up to 46 weeks resulting in a cohort of n=101 individuals, n=296 samples (including n=47 individuals from the cross-sectional cohort).

Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster University ethics committee approved studies with ongoing consent and from NIBTS (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-thaw cycles for all analyses reported within this manuscript.

Clinical information

Basic demographic information and data with regard to probable or definite prior infection with SARS-CoV-2 was obtained from PANDEMIC study participants through the secure online questionnaire requiring responses about positive RT-PCR result and/or time from symptom onset. Anonymised participant samples from USA, SHSCT and NIBTS were provided with age, gender and time since PCR-positive, where a previous test had been carried out.

Laboratory-based immunoassays

Details of laboratory immunoassays are summarised in supplementary methods and Table S1.

UK-RTC AbC-19 LFIA

All analyses were performed on UK-RTC AbC-19 Technical Transfer 3 (TT3) devices at Ulster University according to manufacturer's instructions (details in Table S1). Assays were performed as cohorts, with samples in batches of 10, with one researcher adding 2.5µL of plasma to the assay and a second adding 100µL of buffer immediately following sample addition. After 20 minutes, the strength of each resulting test line was scored from 0-10 according to a visual score card (scored by 3 researchers; Figure S2). A score ≥1 was positive. Details of samples used for analysis for detection of antibodies are available in Supplementary methods.

Statistical analysis

As per Daniel (11) a minimum sample size based on prevalence can be calculated using the following formula: $n = \frac{Z^2 P(1-P)}{d^2}$, where n = sample size, Z = Z statistic for a

chosen level of confidence, P = estimated prevalence, and d = precision. Assuming a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the required sample size at 99% confidence (Z = 2.58) to be 240 individuals. If the true prevalence is lower, 5%, the estimated required sample size given a precision of 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody immunoassays(12).

Statistical analysis was conducted in in R v 4.0.2(13). To assess discordance between test results, data was first filtered to include individuals with an Abbott test result in the range ≥0.25 & ≤1.4, with a 2 x 2 contingency table produced that comprised all possible combinations of [concordant|discordant] test results [within|outside of] this range. A p-value was derived via a Pearson χ^2 test after 2000 p-value simulations via the stats package.

AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To compare test result (Positive|Negative) to age, a binary logistic regression model was produced with test result as outcome – a p-value was then derived via χ^2 ANOVA. To compare time against test result (encoded continuously), a linear regression was performed. We calculated median per time-period and then converted these to log [base 2] ratios against the positivity cut-off for each assay. All plots were generated via ggplot2 or custom functions using base R(14).

Results

We analysed samples from a mixed cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations

and research studies (n=223) and through a convalescent plasma program (n=183). Antibody levels in plasma from these 880 individuals were assessed using the three SARS-CoV-2 immunoassays; EuroImmun IgG, Roche Elecsys IgG/IgM/IgA and Abbott Architect IgG (Table S1, Figure S3). This included a cohort of 223 pre-pandemic plasma samples collected and stored during 2017 to end of May 2019 to determine assay specificity. Of the 657 participants whose samples were collected during the pandemic, 267 (40.64%) previously tested RT-PCR positive with a range of 7-173 days since diagnosis. A total of 225 participants gave time since self-reported COVID-19 symptoms, with a range of 5-233 days from symptom onset, whilst 195 had no symptom or PCR data available. Samples collected in 2020 (n=657) ranged from 19-78 years of age with a median (IQR) of 43 years (±22), and n=454 were female and n=200 male (n=3, not disclosed). Pre-pandemic samples (n=223) ranged from 20-87 years of age with median (IQR) of 50 years (±20) and consisted of n=88 female and n=135 male.

Laboratory based antibody immunoassays

A positive result for antibody on one or more of the three laboratory immunoassays was recorded for 385/657 (58.6%) participants who provided a sample during the pandemic. By EuroImmun ELISA, 346 were positive, 20 borderline and 291 were negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott determined 310 positive and 347 negative (Table S2, Figure S3). The median age across all age groups combined was lower for participants testing positive across each of the immunoassays (median [sd] for positive versus negative, respectively: EuroImmun, 41 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41 [13.18] vs 47 [13.09]). (Figure S4, $p<0.0001$). When segregated by age group, however, differences were less apparent in certain groups (Figure S5). Excluding the

pre-pandemic cohort, this gap reduced but remained statistically significant EuroImmun, 41 [13.18] vs 45 [12.49]; Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41 [13.26] vs 44 [12.63]) ($p<0.01$) (median [sd] for positive versus negative). Of note, out of 267 individuals with a previous positive RT-PCR result for SARS-CoV-2 viral RNA, 14 (5.2%, Figure S3a) did not show detectable antibodies by any of the three immunoassays, with no association found with age, gender or time between test and blood draw (data not shown).

The three commercial laboratory immunoassays provide a ratio value that increases with IgG antibody titre. When correlation between these values is assessed, good overall agreement is observed between the three immunoassays (Figure 1, Figure S5). As highlighted by Rosadas *et al*., we also see significant disagreement in the Abbott 0.25-1.4 range when compared to EuroImmun and Roche (Figure 1a,b; chi-square p-values: EuroImmun vs Abbott, $p<0.001$; Roche vs Abbott, $p<0.001$)(15).

Duration of humoral response to SARS-CoV-2

In a cross-sectional analysis of antibodies over time, we found IgG antibodies could still be detected in individuals (excluding pre-pandemic) across all three immunoassays used up to week 20 (day 140) (Figure 2). We note a statistically significant decrease in signal with respect to time across each assay (p-value [estimate slope]): EuroImmun, $p=0.028$[-0.823]; Roche, $p=0.002$ [-0.125]; Abbott, $p<0.0001$ [-3.673]. These remained statistically significant after adjustment for age. Antibody levels (expressed as a ratio of median result per timepoint divided by positivity cut off; Table 1) peaked at Week 1-2 for EuroImmun (1.33) and Abbott (1.64), though reached highest levels at Week 8-12 when measured by Roche (5.45). By week 21-24, median score for all tests had dropped below the positivity

cut off, though a small number of samples remained above the positive cut off at these later timepoints (Figure 2).

Samples from the NIBTS convalescent plasma program continued to be collected throughout 2020-early 2021. A total of n= 897 samples from n=676 individuals were collected, 744/883 tested by EuroImmun were positive (>1.1, with values range of 0.051-34.361), 556/749 tested by Abbott were positive (>1.4, with values ranging from 0.01-8.85). Individuals with a positive RT-PCR result and a EuroImmun result >6 were sequentially sampled (with median 3, range 2-9 samples per individual) and analysed by both EuroImmun (n=101 individuals), and Abbott immunoassays (n=75 individuals). Median age (IQR) for this cohort is 51 years (±21) with a range from 18-70 years and n=27 female, n=74 male. Longitudinal analysis shows persistence of detectable IgG antibodies until up to 302 days (43 weeks) by Abbott immunoassay (at which point this assay was discontinued at NIBTS) and 323 days (46 weeks) by EuroImmun ELISA, with a gradual decline over time (Figure S6). None of the individuals who were initially positive by Euroimmun SARS-CoV-2 S1 IgG assay dropped to below the EuroImmun positivity threshold (>1.1) over the course of the follow-up while 26 who were initially positive by Abbott SARS-CoV-2 NP IgG fell below the Abbott threshold (>1.4).

UK-RTC AbC-19

Using the commercial immunoassays described we established a well characterised serology sample set of ‘known positive’ and ‘known negative’ for IgG antibodies to SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.

AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore required all samples to be positive by the EuroImmun SARS-CoV-2 IgG ELISA, which

likewise detects antibodies against the S1 domain (16). To develop this characterised cohort, samples were also required to be positive by a second immunoassay (Roche or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG antibody, we assessed 350 plasma samples from participants classed as 'known negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from individuals confirmed to be negative across all three laboratory assays (Roche, EuroImmun, Abbott). Using these positive n=304 and negative n=350 antibody cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the AbC-19 LFIA (Table 2).

Given a recent report of lower specificity in the AbC-19 LFIA (17) and the possibility of introducing sample bias, we revised our inclusion criteria for the negative cohort. For the pre-pandemic cohort, we included samples from all 223 individuals, regardless of results on other laboratory immunoassays. When this assumed negative pre-pandemic cohort was used for laboratory evaluation for target condition of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 2). We expanded the negative cohort to include all samples that matched our criteria (samples collected during the pandemic to be negative by all three laboratory assays and all pre-pandemic samples regardless of other immunoassay results). The specificity observed on this extended negative cohort of 488 samples was 99.59% (98.53% to 99.95%, Table 2). For sensitivity analysis on a positive cohort (samples positive by EuroImmun and one other test), we were able to analyse all samples previously untested due to limited testing capacity and tested a positive cohort of 330 samples giving a sensitivity of 97.58% (95.28% to 98.95%, Table 2). When we sorted samples analysed in both negative (n=488) and positive cohorts (n=330) by

RT-PCR status and assessed AbC-19 LFIA sensitivity by including only those that were RT-PCR positive (n=227), the test showed a sensitivity of 92.07% (87.76%-95.23%, Table S3). However, of the n=18 RT-PCR positive individuals negative for IgG antibodies by AbC-19, n=12 showed no detectable antibodies by all three laboratory assays (EuroImmun, Roche or Abbott), suggesting that antibodies are not present in those samples.

When used as intended by the public, the AbC-19 LFIA provides binary positive/negative results. However, when assessing LFIA in the laboratory, each test line was scored against a scorecard by three independent researchers (0 negative, 1-10 positive; Figure S2). When compared to quantitative outputs from the Abbott, EuroImmun and Roche assays, the AbC-19 LFIA shows good correlation (Abbott r=0.84 [p<0.001]; EuroImmun r=0.86 [p<0.001]; Roche r=0.82 [p<0.001]; Figure 3, Figure S7-Figure S9).

Analytical specificity and sensitivity of AbC-19 LFIA

We observed no cross-reactivity across samples with known H5N1 influenza, Respiratory syncytial virus, Influenza A, Influenza B, Bordetella Pertussis, Haemophilus Influenzae, Seasonal coronavirus NL63 and 229E on the AbC-19 LFIA (n=34 samples, n=8 distinct respiratory viruses; Table S4). Against a panel of external reference SARS-CoV-2 serology samples, the AbC-19 LFIA detected antibodies with scores commensurate to the EuroImmun ELISA scores (Figure S10, Table S5).

Discussion

Serological antibody immunoassays are an important tool in helping combat the SARS-CoV-2 pandemic. The duration of the humoral immune response is of particular importance, to inform an individual's protection following both natural infection and vaccination. Using a large cohort of individuals across a wide age range (18-78), we assessed antibody levels across up to three laboratory immunoassays perform a cross-sectional and longitudinal analysis over time. Our results show strong correlation between all three immunoassays, with shortcomings in the Abbott system output 0.25-1.4 range, as described previously, suggesting an overestimated positive cut-off (Figure 1) (15).

Longitudinal studies on SARS-CoV-1 convalescent patients suggests that detectable IgG can still be present as long as 2 years after infection (18). There are conflicting reports of the longevity of the humoral response to SARS-CoV-2 infection which differ in the make-up of the cohort studied, the assays used, and the length of time since symptom onset. To our knowledge, this study represents the longest follow-up period with detection of IgG antibodies to both spike and nucleocapsid protein more than 10 months after RT PCR positive status (and beyond in a small number of samples, Figure 2, Figure S6). In this study, samples were collected through a convalescent plasma program (Figure S6), with individuals selected for sequential plasma donation based on an initial high EuroImmun assay score. In contrast to the time series analysis of healthcare workers recruited prospectively by Manisty *et al*., we observed no cases where Euroimmun ELISA-measured anti-Spike antibody levels fell below threshold, whilst a large number of Abbott measured anti-Nucleocapsid antibody levels dropped below the positivity threshold (34.7% 26/75). However, this may be an overestimate given the shortcomings of the Abbott assay

described above (Figure 1) (19). In a similar longitudinal study of 51 symptomatic participants, Dan *et al*. estimated that half-life ($t_{1/2}$) for IgG-Spike (103 days) was longer than that for IgG-Nucleocapsid (68 days), although with a considerable overlap of 95% confidence intervals (20).

In our more diverse cross-sectional cohort, we also note a statistically significant decline over time but levels remain detectable at 140 days (Figure 2). We note that IgG levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as Week 8-12 from first symptoms or a viral RNA RT-PCR positive result, though this may be an artefact of lower number of participants at earlier timepoints (Table 1). Robust antibody responses are produced in our cohorts across a wide age range (18-78 years old, Figure 2, Figure S6). We detect a slightly but significantly lower median age of participants testing positive (Figure S4); however, this is likely be due to cohort characteristics and not a true reflection of the population or indication of test performance.

A difficulty faced in validation of antibody diagnostic assays has been access to samples with known SARS-CoV-2 antibody status. As previously described, there is no clear gold standard reference against which to assess SARS-CoV-2 immunoassays. A positive RT-PCR test has been used previously to indicate previous (COVID-19) SARS-CoV-2 infection, though this approach is limited by a high rate of false negatives and positives in RT-PCR testing, failure in some cases to develop IgG antibodies (sero-silence or lack of antibody against the same antigenic component of the virus as the immunoassay uses as a capture antigen) and the lack of RT-PCR testing availability early in the pandemic (3,5,21). SARS-CoV-2 IgG antibodies were undetectable in 14 of 267 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA

positive participants in this study. It is unclear if this is due to insufficient/absent antibody production in these individuals at the time the sample was taken, or due to a false positive PCR result which may occur in the UK at a rate between 0.8- 4.0% (6). Self-assessment of symptoms for COVID-19 (disease) is a poor indicator of previous infection, even amongst healthcare workers (22). Additionally, the kinetics of a SARS-CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus with time, contributing to false negative RT-PCR test results for individuals who may be late to present for virus detection tests (5,23).

To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-CoV-2 antibody in a laboratory evaluation, we developed a reference standard for SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar approach was used in a recent seroprevalence study in Iceland, whereby two positive antibody results were required to determine a participant sample as positive for SARS-CoV-2 antibody (21). Our evaluation of performance metrics for the UK-RTC AbC-19 LFIA to detect antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59% specificity. In an evaluation of the AbC-19 tests, Mulchandani *et al*. observed a specificity of 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report a sensitivity of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a previous RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys antibody test, which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid portion of SARS-CoV-2 (22). In RT-PCR positive individuals from our cohorts, the AbC-19 test showed a similar sensitivity (92.07%, 87.76%- 95.23%, Table S3). However, we demonstrate the drawbacks of this approach given that in 12 of 18 AbC-19 false negatives, none of the four immunoassays used (EuroImmun, Roche, Abbott

or AbC-19) detected antibodies, suggesting either a false RT-PCR result, a failure to produce IgG antibodies or sero-reversion before sample collection in these individuals. Another recent evaluation of the AbC-19 LFIA by Moshe *et al*. determined a sensitivity of (100% (98.1-100%) on laboratory sera, using a composite reference standard of antibody positive by either Spike protein ELISA or hybrid DABA assay and specificity of 99.8% (98.9-100%) against pre-pandemic samples. However, when AbC-19 performance was analysed on matched finger-prick and serum samples against the same antibody standard, a lower sensitivity was observed (finger-prick 69% (53.8-81.3%), serum 92% (80-97.7%)) (24).

In our study, strong correlation was observed in quantitative score between results on all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA (Figure S8, S9). This is to be expected, given both the AbC-19 LFIA and EuroImmun ELISA detect IgG antibodies against spike protein. Importantly, for the assessment of immunity to prior natural infection as well as to immunisation, IgG antibodies against SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA and AbC-19 LFIA are known to correlate with neutralizing antibodies, which may confer future immunity (20,25,26). Previous evaluations of sensitivity and specificity reported by Public Health England (PHE), showed a EuroImmun sensitivity of 72% and specificity of 99%, Abbott with sensitivity of 92.7% and specificity of 100% and Roche with sensitivity of 83.9% and specificity of 100% (27–29). The PHE analyses for each of these tests used previous infection (RT-PCR positive status) as a reference standard, the limitations of which are discussed above.

In the use of characterised 'known positive' and 'known negative' cohorts, one limitation of this study is its potential for spectrum bias, whereby our positive-by-two reference system may artificially raise the threshold for positive sample inclusion, possibly resulting in the overestimation of the sensitivity of any test evaluated (30). However, similar issues have been raised when using previous RT-PCR result or definitive COVID-19 symptoms as inclusion criteria given these will likely skew a cohort towards more severe disease, especially given issues of RT-PCR availability outside of hospital settings during the first wave (5). Importantly, our mixed origin of samples forming the cohort provides a positive cohort for assessing assay sensitivity that includes individuals from the general public, healthcare workers and from convalescent plasma programmes. In the absence of a clear gold standard test, our system relies on no single test (each with their individual shortcomings) and instead takes an average of three. Our analysis of specificity on only pre-pandemic individuals (n=223) shows similar specificity (99.55%) to the larger mixed 'known negative cohort' (n=488, sensitivity 99.59%). We also demonstrate a high level of analytical specificity of the AbC-19 test with no cross-reactivity against a panel of other respiratory viruses, including SARS-CoV-1 NL63 and 229E (Table S4).

Our assessment of the AbC-19 LFIA in a laboratory setting, using characterised cohorts of known SARS-CoV-2 antibody positive and antibody negative plasma, shows good performance metrics for its ability to detect SARS-CoV-2 IgG antibodies following natural infection. We note our use of plasma from venous blood samples, as opposed to a finger prick blood sample as would be used in rapid testing scenarios (24). Additionally, when the AbC-19 LFIA was used on our cohort, a number of the positive results scored low (1/10 using the score card under laboratory conditions,

Figure 3), with a faint test band visible to a trained laboratory scientist but perhaps difficult to identify as positive by individuals performing a single test (Figure S10). This faint line may be reflective of the longer time from infection for the Northern Ireland cohort used. If the AbC-19 LFIA is to be used in clinical settings it is important to determine if all users observe the same results as observed in this laboratory evaluation.

This assessment of the AbC-19 LFIA does not provide data on how this test will perform in a seroprevalence screening scenario, but instead provides metrics for the performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as opposed to previous COVID-19 infection. An important potential use of the AbC-19 LFIA would be in monitoring the immune response to vaccination, with most vaccines utilising SARS-CoV-2 Spike protein antigens (31).

Conclusion

We present a comprehensive analysis of pre-pandemic and two large pandemic cohorts (more than 700 individuals) and in a longitudinal analysis showing that IgG antibodies to SARS-CoV-2 antigens are detectable more than 10 months from positive RT-PCR test. We use antibody positive status as an alternative to RT-PCR positive status as a standard for assessing SARS-CoV-2 antibody assays and show strong performance for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-CoV-2 antibodies. User experience in future studies in the real world is important and may alter the performance characteristics. Also, the effect of operator training will have direct effects upon test performance. We welcome further clinical evaluation of the AbC-19 LFIA in large cohorts of symptomatic and asymptomatic individuals alongside

large studies assessing vaccination outcomes in individuals to fully validate its implementation across all intended use cases.

Declarations

Ethics approval and consent to participate

All study participants provided informed consent. This study was approved by Ulster University Institutional Ethics committee (REC/20/0043), South Birmingham REC (The PANDEMIC Study IRAS Project ID: 286041Ref 20/WM/0184) and adhered to the Declaration of Helsinki and Good Clinical Practice.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Consent for publication

Not applicable.

Dissemination to participants and related patient and public communities.

Links to this work will be included on the study website (https://www.ulster.ac.uk/coronavirus/research/research-output/pandemic-study) and participants will be alerted that the work has been published.

Data sharing

Data are available on reasonable request to the corresponding author.

Competing interests:

At the time of this study TM and JML acted as advisors to CIGA HealthCare, an industrial partner in the UK Rapid Test Consortium. No personal financial reward or renumeration was received for this advisory role. At the time of submission of this manuscript TM and JML no longer held these advisory positions.

All other authors have no potential conflict of interest to report.

Funding:

Costs for assays and laboratory expenses only will be paid by UK-RTC as is normal practice (UU-UK-RTC-2020-001). The authors have not been paid or financially benefitted from this study.

The advisory roles within CIGA Healthcare were unpaid temporary roles. This manuscript and associated data within this paper has only been used to build confidence into the overall device design and performance assessment of the UK RTC AbC-19 devices and such work was never commissioned for any government contractual consideration.

Authors' contributions:

TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR, SM and KYN analysed data, KB performed all statistical analyses/interpretations and produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and provided Blood Transfusion cohort samples. TM, RP and AN coordinated participant recruitment, consent and sampling. WB and JML developed online consent forms, questionnaires and databases. LR, JM, AK, AA, GEW, DH, SS, CCS performed sample collection and processing. LR and TM wrote the manuscript, with significant contributions from JM, AN and KB. All authors reviewed and approved the final manuscript.

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Table 1: Antibody level ratios for assays over time

Antibody level ratios for assays over time show varying peak levels depending on test. Calculated by first establishing the median per time period, then calculating log2 ratio for each period versus each respective assay positivity cut-off.

	Ratio median antibody level: assay positivity cut-off									
	Week									
	Pre-2020	1-2	3-4	5-8	9-12	13-16	18-20	21-24	25-28	29+
EuroImmun	-2.65	1.33	0.2	0.95	1.32	0.47	0.04	-2.01	-2.26	-2.01
Roche	-3.64	3.16	3.05	5.20	5.45	4.14	4.42	-3.54	-3.69	-3.61
Abbott	-5.54	1.64	-0.51	1.12	0.86	0.08	-0.59	-5.13	-5.13	-6.13
Sample number (n=)	223	20	10	52	90	202	53	11	12	11

Table 2: UK-RTC AbC-19 LFIA performance metrics against known antibody positive and known antibody negative cohorts.

Total Negative	True Negative	False Positive	Total Positive	True Positive	False Negative	Sensitivity % (95 CI)	Specificity % (95 CI)
Pre-pandemic (n=223)							
223	222	1	n/a	n/a	n/a	n/a	99.55% (97.53% to 99.99%)
Initially reported cohorts (n=654)							
350	350	0	304	297	7	97.70% (95.31%-99.07%)	100.00% (98.95%-100.00%)
Extended cohorts (n=818)							
488	486	2	330	322	8	97.58% (95.28%-98.95%)	99.59% (98.53%-99.95%)

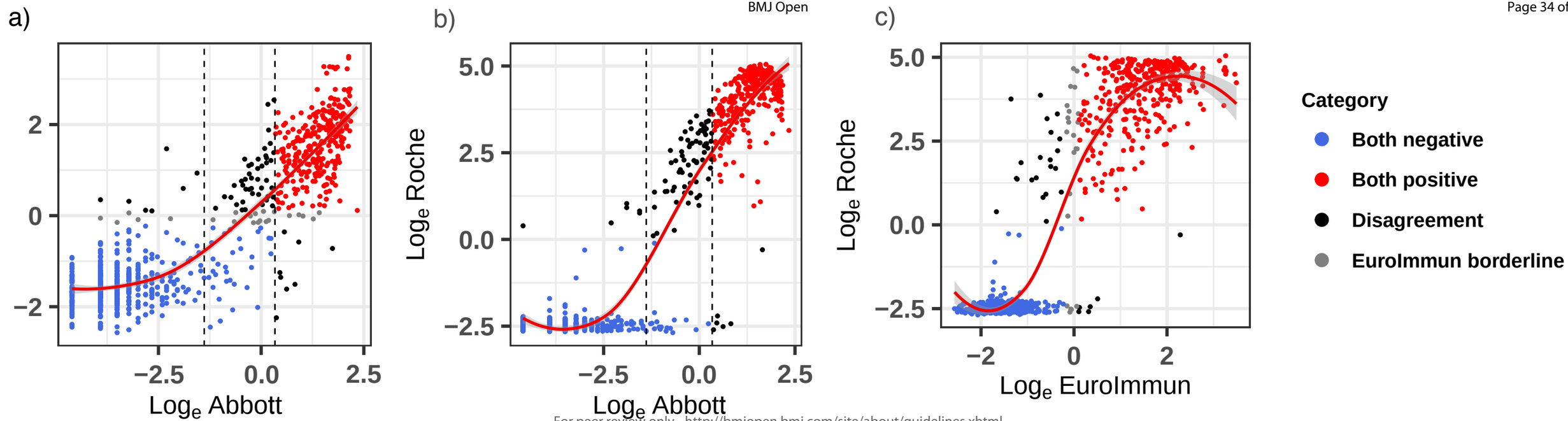
Figure Legends

Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott and c) Roche immunoassays. Pearson χ^2 test was used to assess correlations. The results for each test were log transformed to ensure results follow a normal distribution. Negative agreement shown as blue dots, red dots show positive agreement for the two immunoassays, whilst black dots show disagreement and grey dots as the EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4. n=880. The graphs show positive correlations between all immunoassays evaluated, with the fewest disagreement of results between the Log of Roche and the Log of EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.

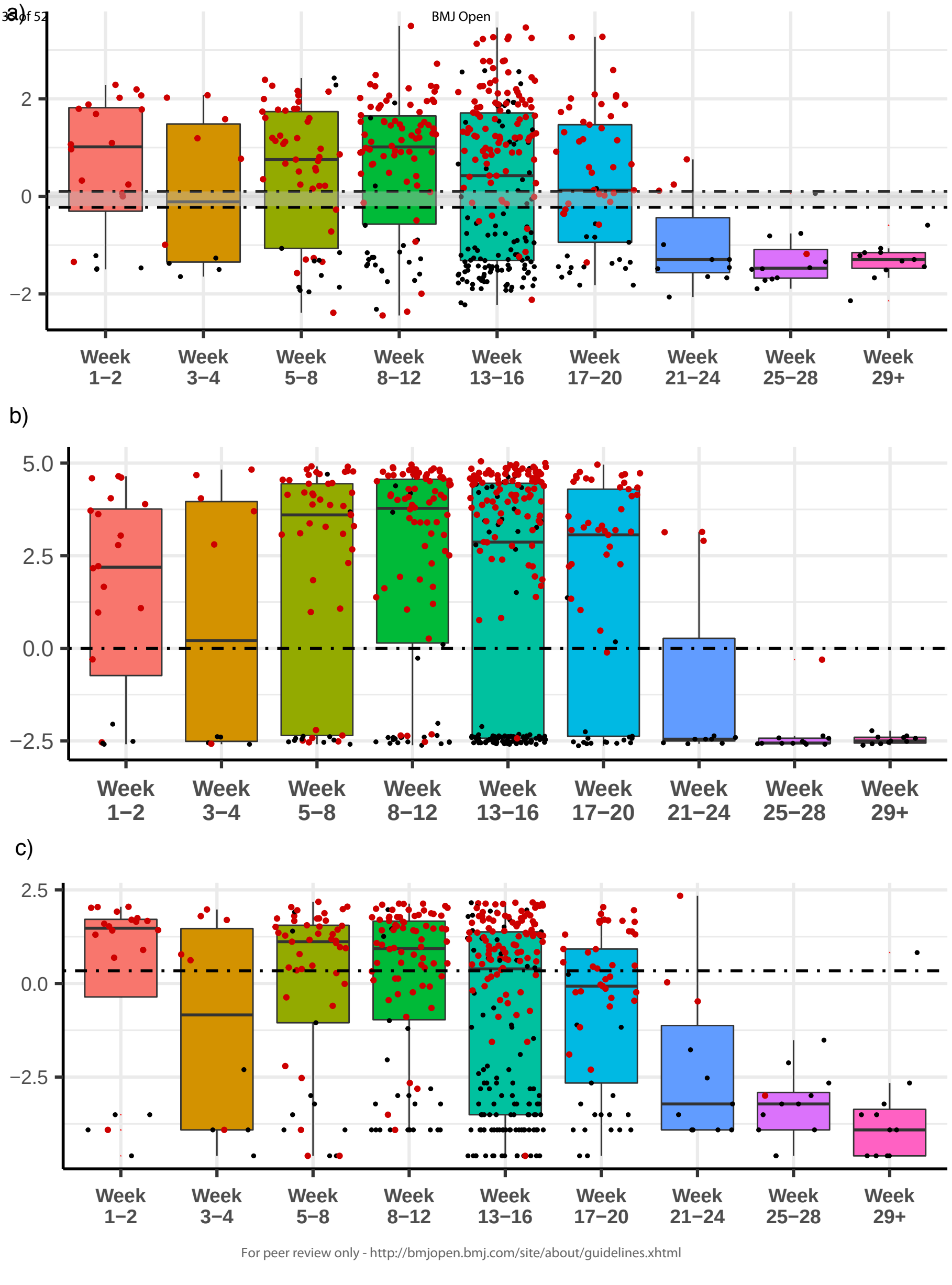
Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c) Abbott, relative to weeks since first reported symptoms or positive PCR result (where data available, n=685). RT-PCR positive individuals are denoted by red dots, while individuals with time since symptom data are denoted in black. Dashed lines delineate $\log_e$ equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result between the two lines). Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).

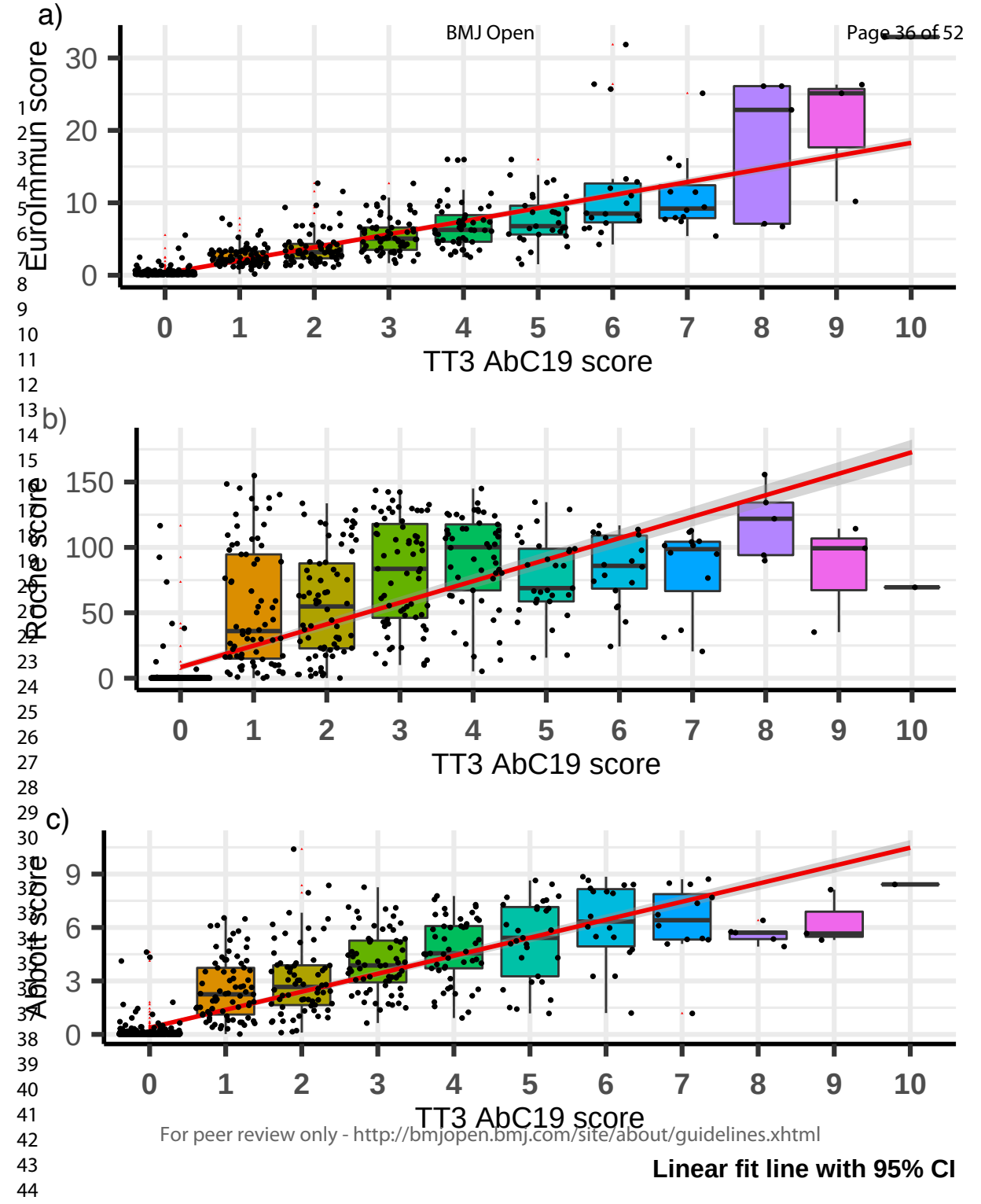
Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing AbC-19 TT3 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line

of best fit with 95% confidence interval shaded in grey. Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).



Fit lines, LOESS with 95% CI
Vertical lines mark Abbott test range 0.25−1.4


a)
Log$_e$ EuroImmun
2
0
−2
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+
b)
Log$_e$ Roche
5.0
2.5
0.0
−2.5
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+
c)
Log$_e$ Abbott
2.5
0.0
−2.5
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+



Linear fit line with 95% CI

Supplementary Materials

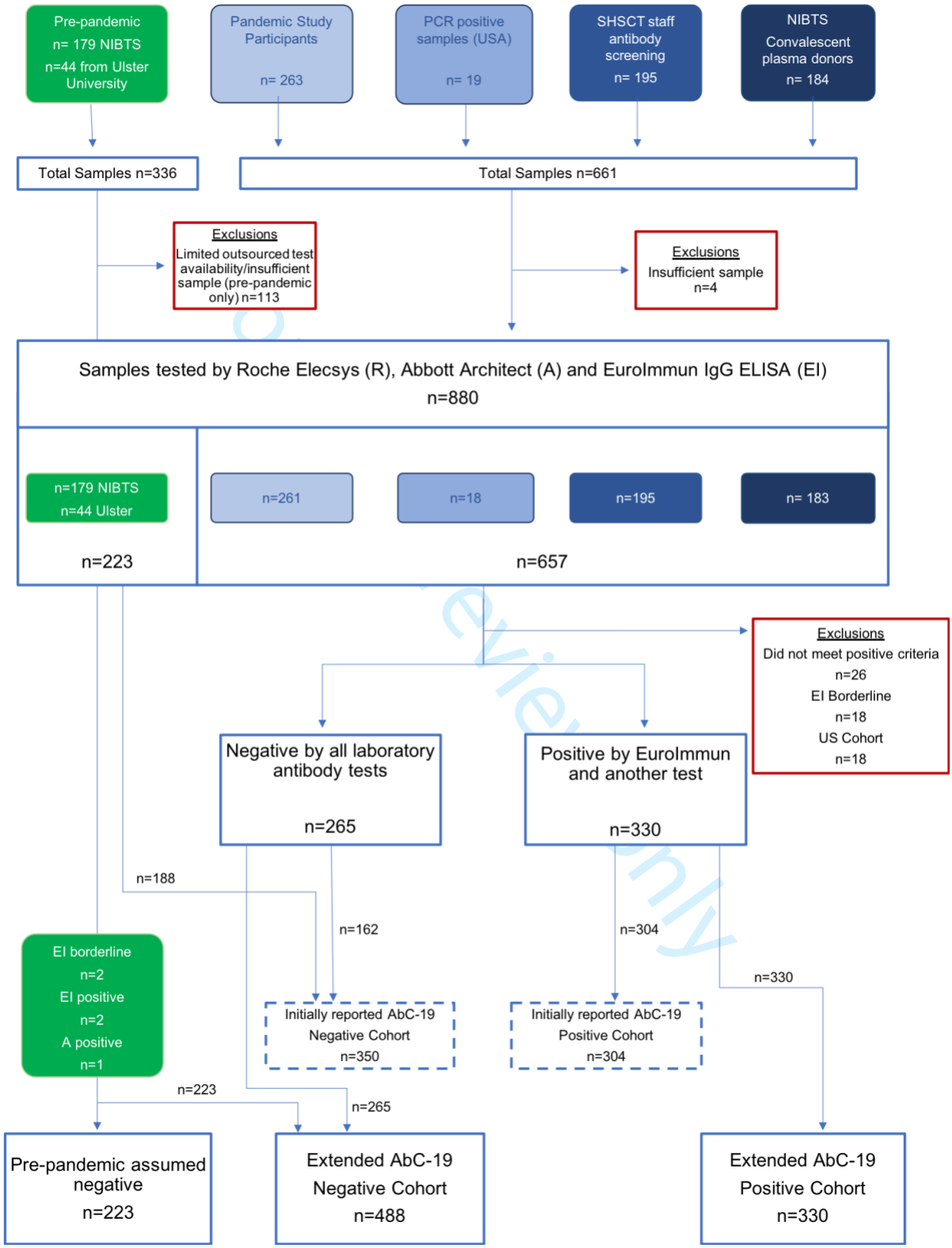
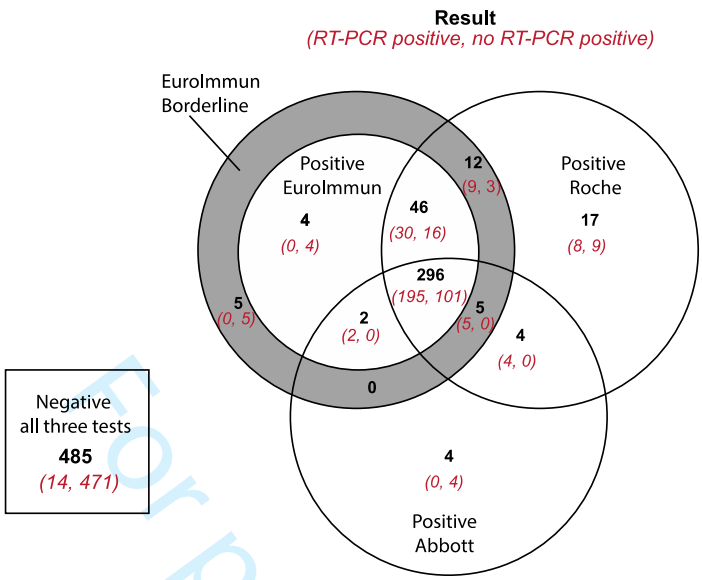


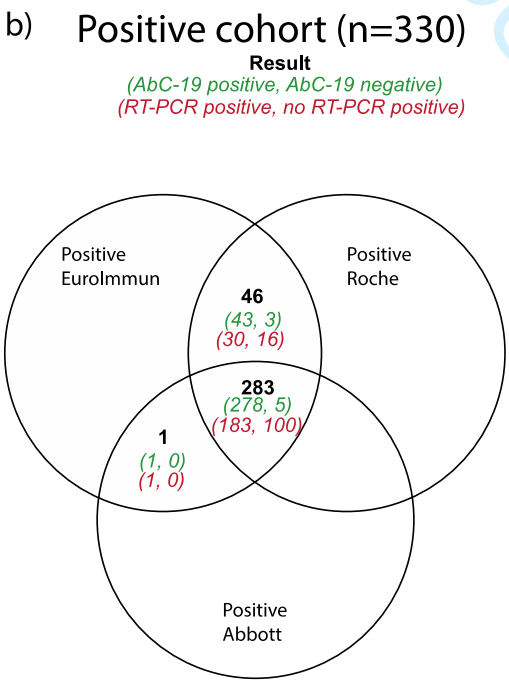
Figure S1: Flow of participant plasma samples through cross-sectional study. All available samples from participants within each cohort, and the included and excluded samples at all stages. Freeze thaw cycles were closely monitored for all sample aliquots. Pre-pandemic samples taken forward for Roche, Abbott and EuroImmun testing were selected based on aliquot volume and availability.

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

a)



b)



c)

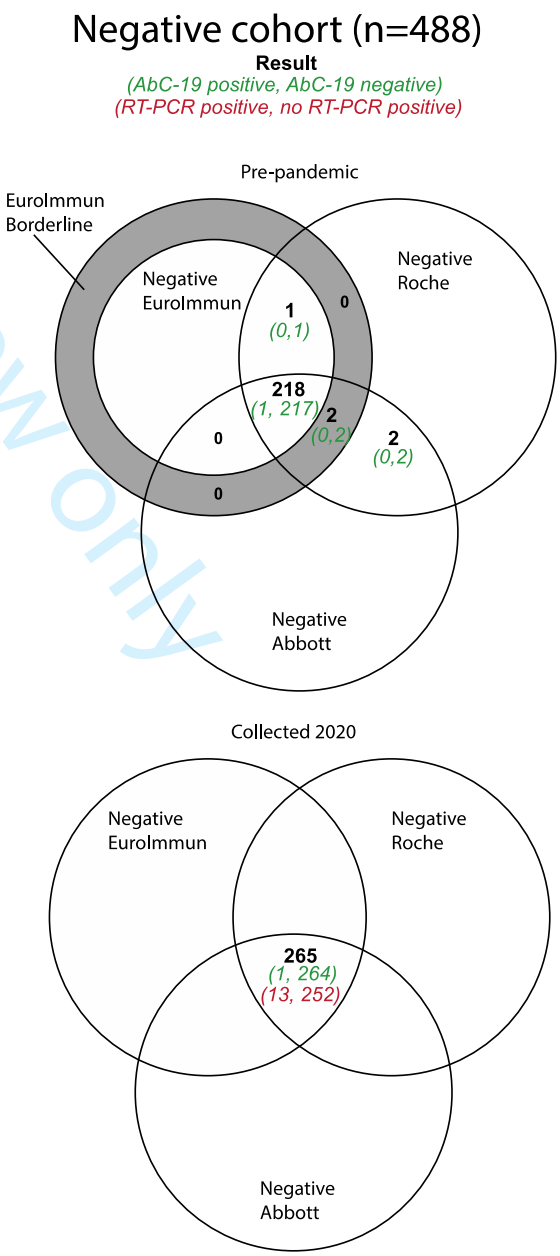


Figure S3: Venn diagrams demonstrating result overlap between laboratory assays in a) the initial immunoassay cohort (n=880), b) the positive and c) negative cohorts assessed with AbC-19 TT3. Result in each circle overlap in bold, (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below.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

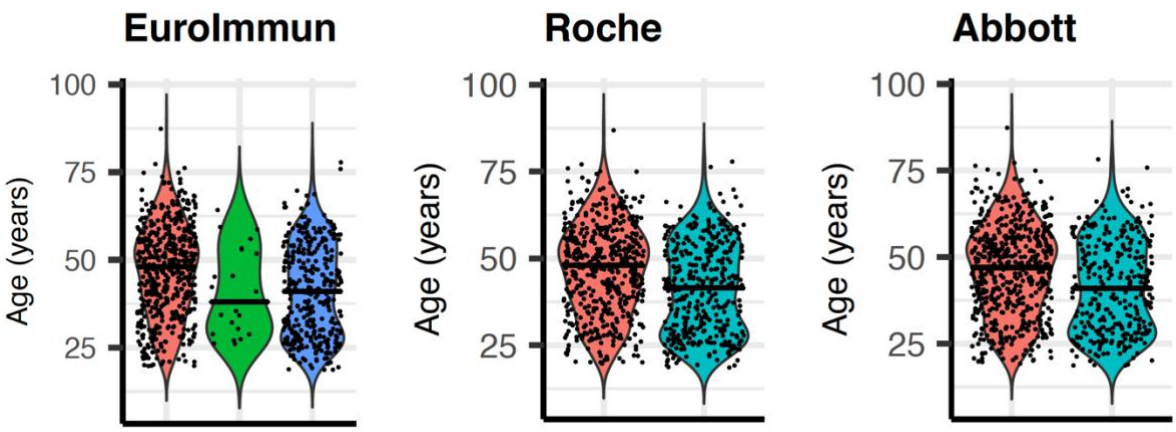


Figure S4: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.

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

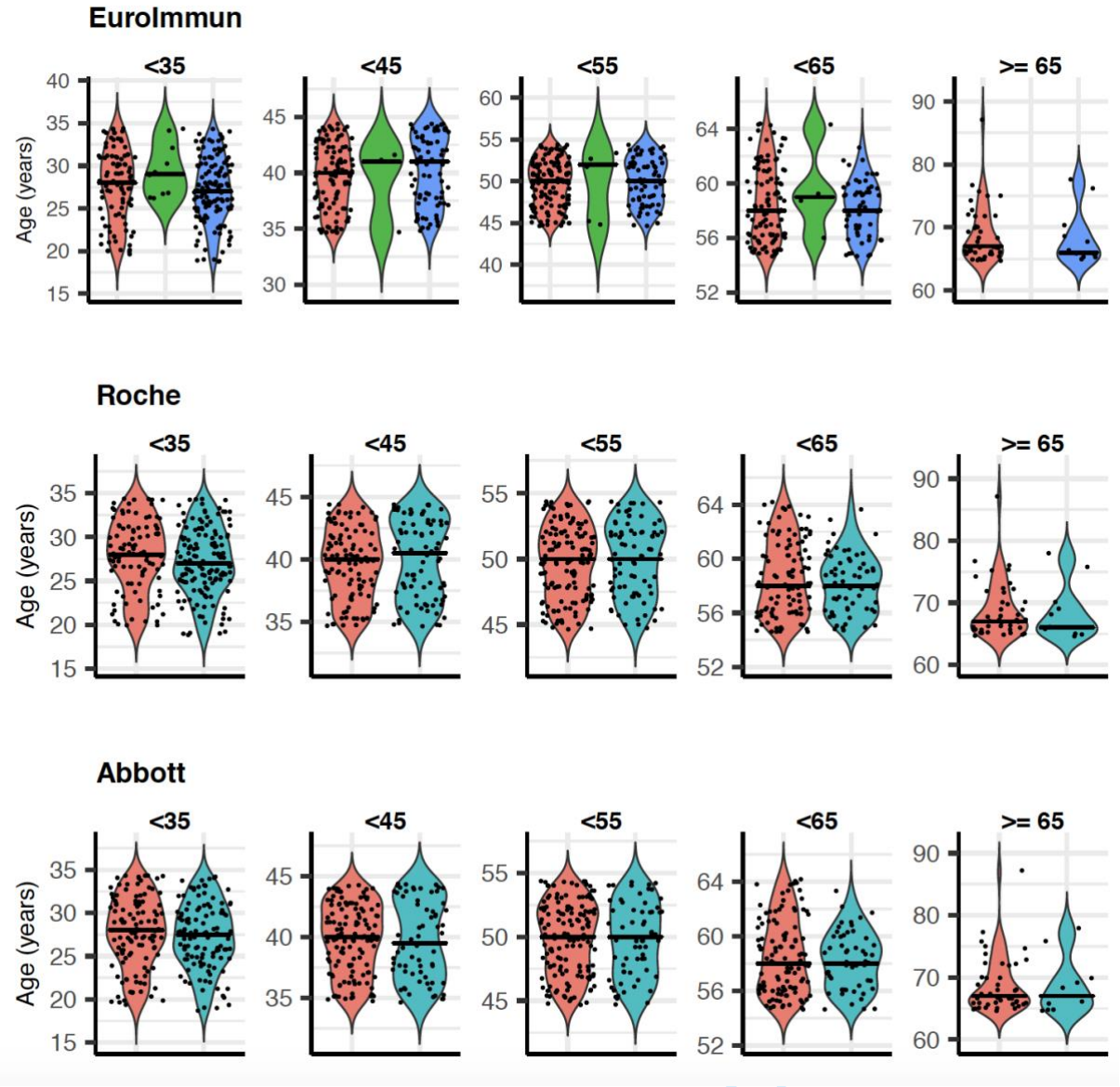


Figure S5: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.

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

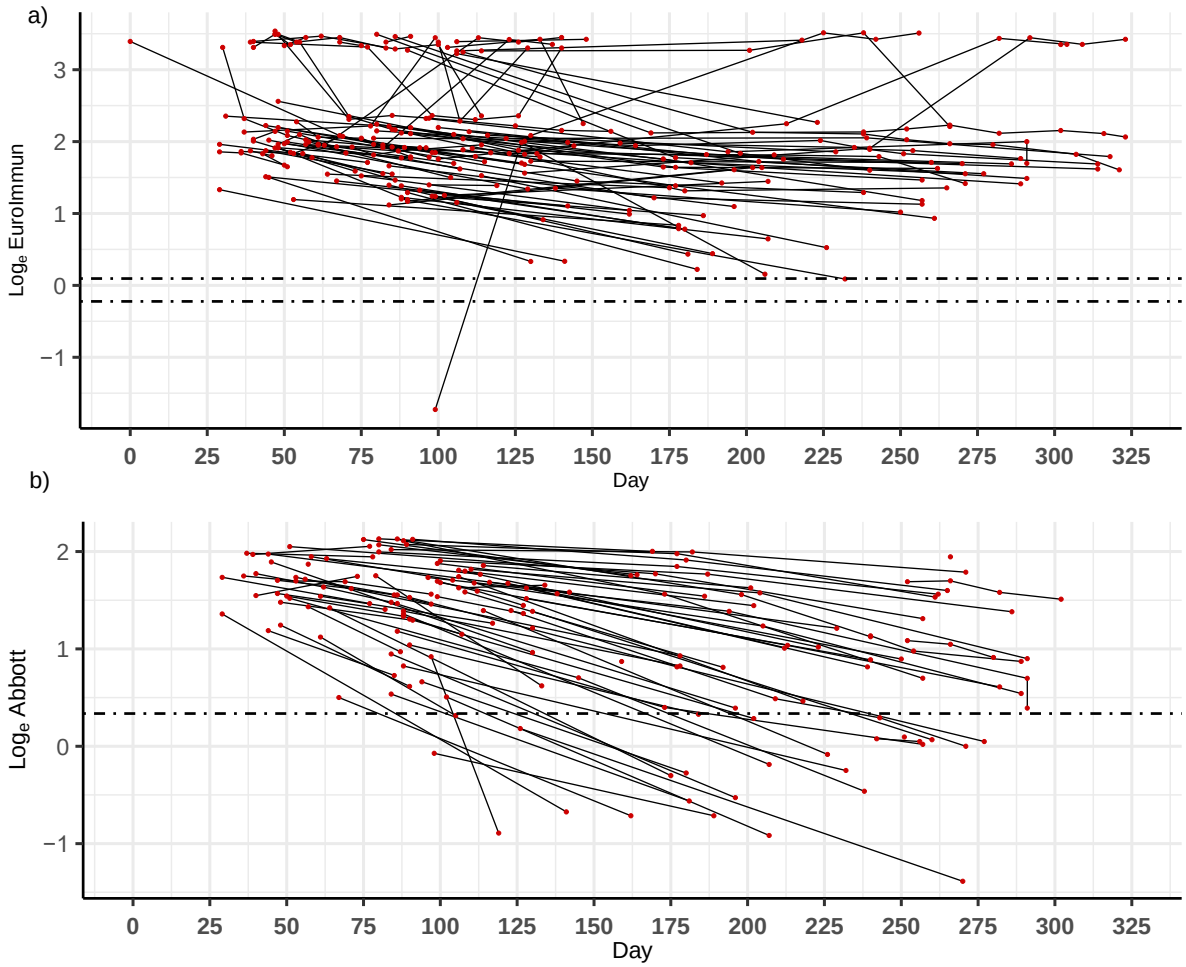


Figure S6: Longitudinal analysis of convalescent plasma donor sequential samples (2-9 samples per individual) by a) EuroImmun ELISA or b) Abbott immunoassay. a) n=101 individuals, grey shading indicates borderline region, upper dotted line indicates positivity threshold (1.1), lower dotted line indicates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

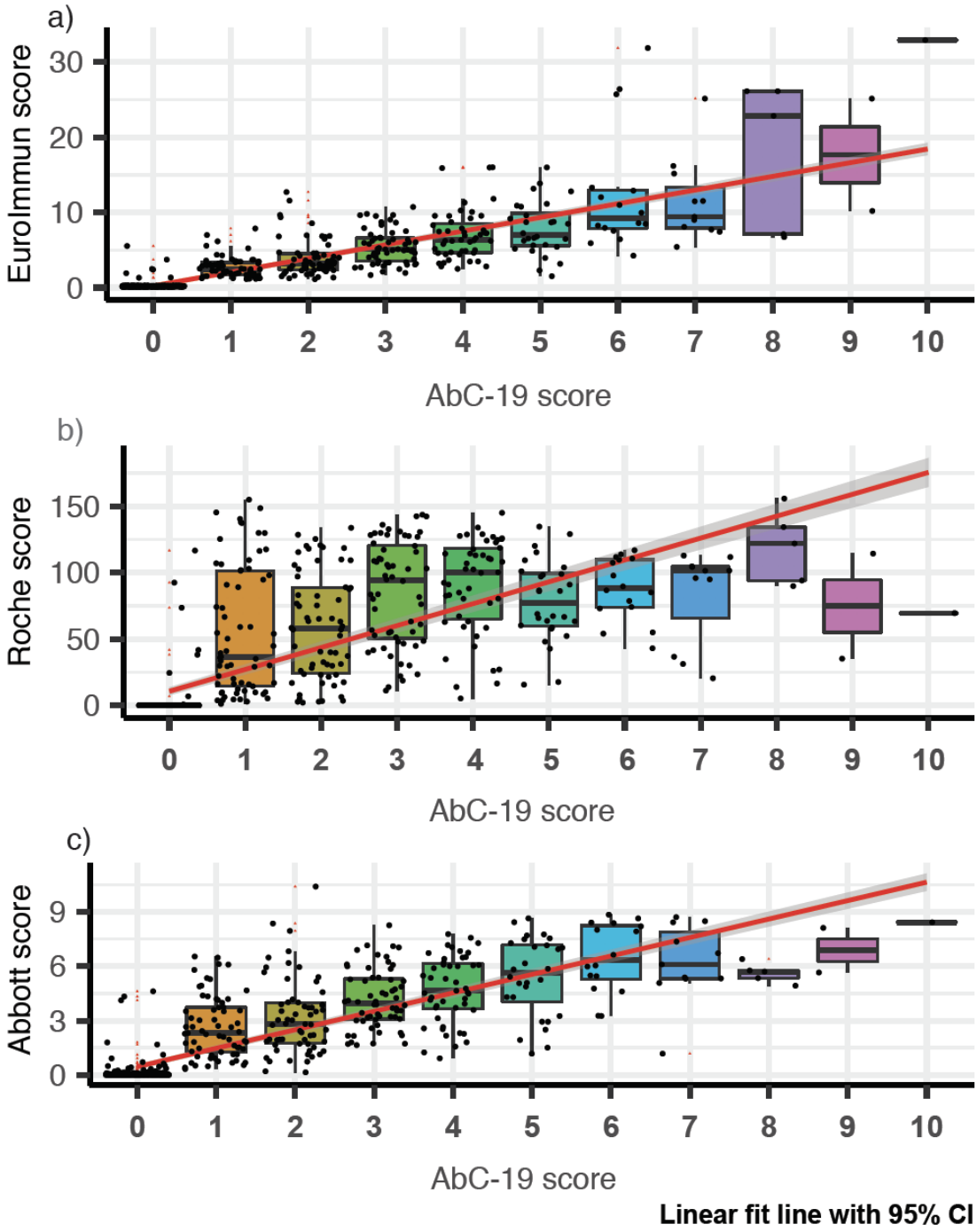


Figure S7: AbC-19 initially reported cohort n=654 correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing TT3 AbC-19 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line of best fit with 95% confidence interval shaded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

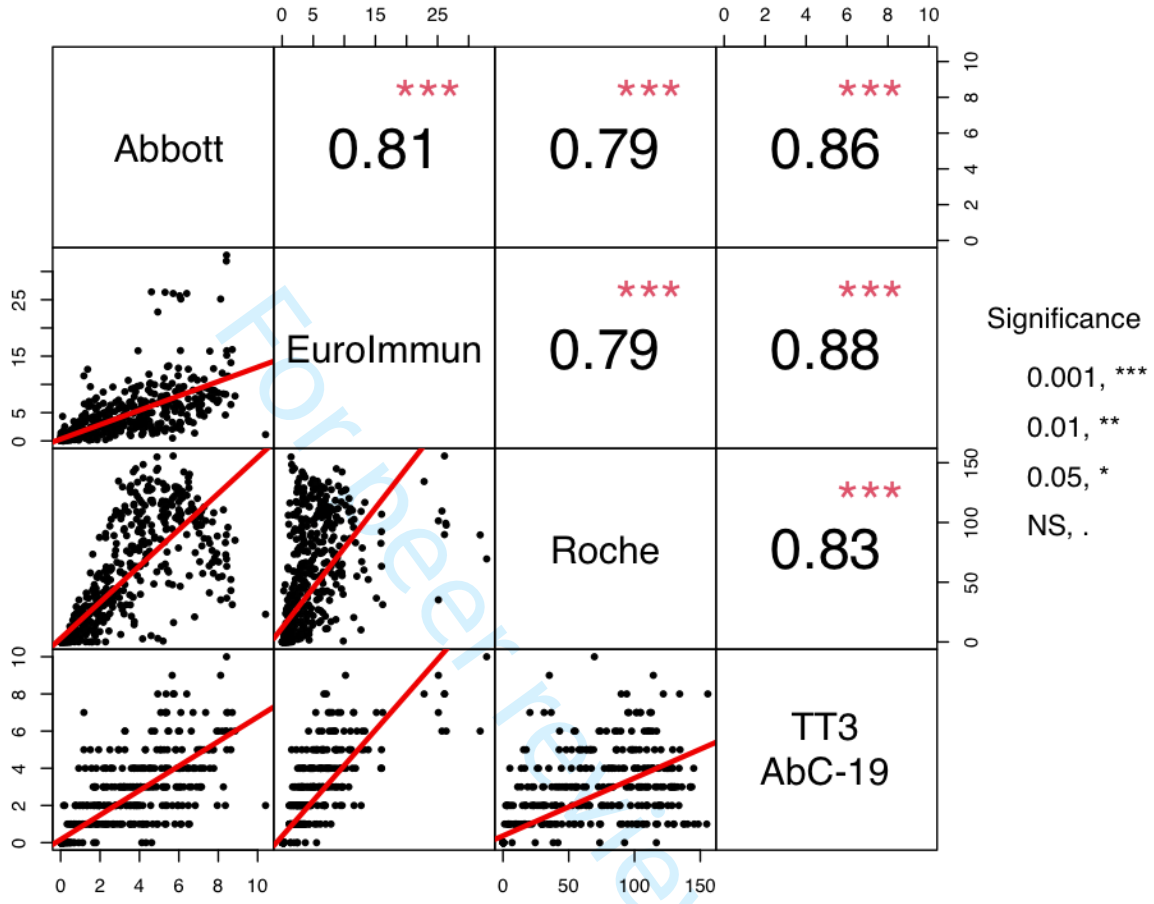


Figure S8: **Correlation matrix between Abbott, EuroImmun, Roche and initially reported AbC-19 cohort (n=654) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly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

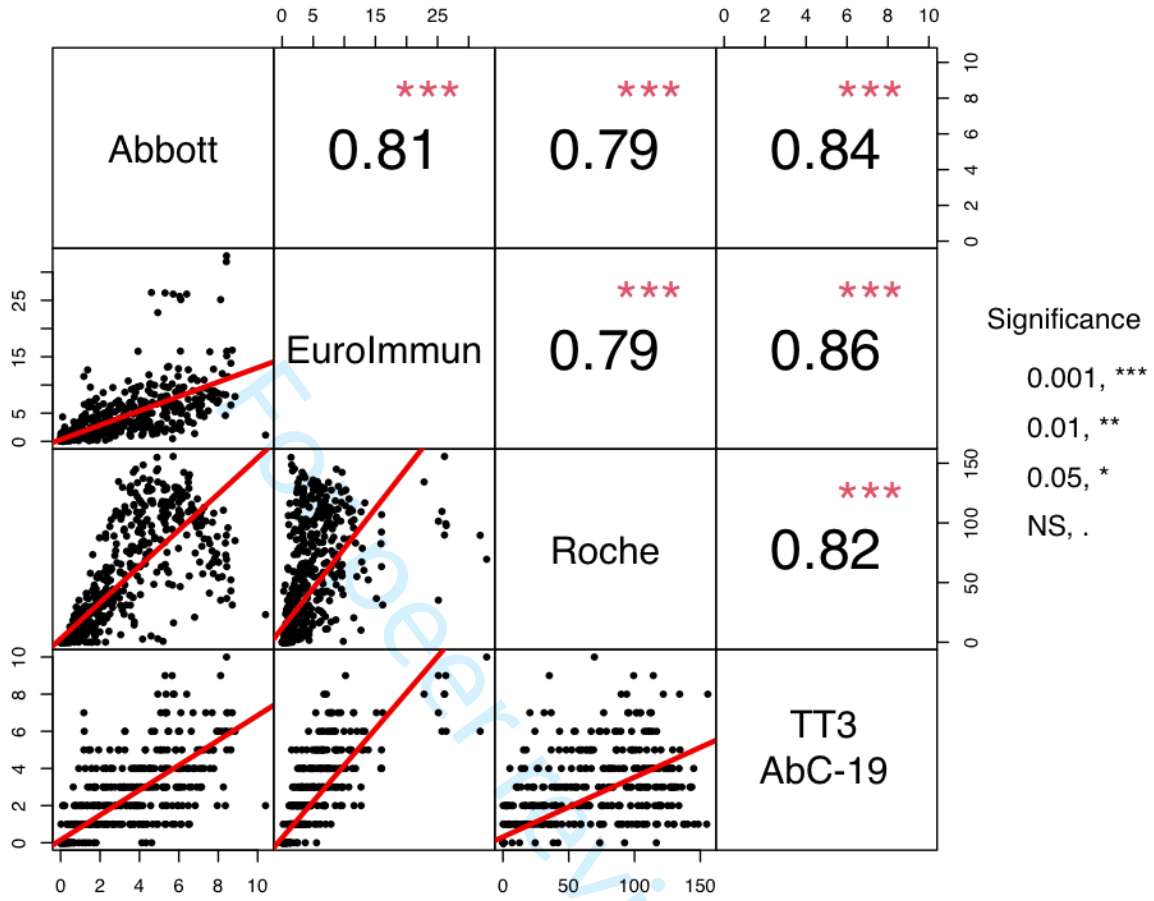


Figure S9: **Correlation matrix between Abbott, EuroImmun, Roche and extended AbC-19 cohort (n=818) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly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

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

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

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

Immunoassay	Principle	Antigen Target	Assay Time (min)	Antibody Detected	Measurement	Result	Calibration	Evaluation of results	Results
EuroImmun ELISA	Enzyme-linked immunosorbent assay (enzyme-HRP)	S1 domain of the spike protein	120	IgG	Photometric measurement of the color intensity using wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm	OD (Optical density)	One Positive calibrator	OD of clinical sample/OD of calibrator	< 0.8 Negative, ≥ 0.8 to <1.1 Borderline, ≥ 1.1 Positive
Roche Elecsys immunoassay	Electro-chemiluminescence	Nucleocapsid	18	IgG, IgA and IgM	Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier	RLU (Relative Light Intensity)	One Positive calibrator and one Negative calibrator	The analyzer automatically calculates the cut-off based on the measurement of ACOV2 Cal1 (negative) and ACOV2 Cal2 (positive). The result of a sample is given either as reactive or non-reactive as well as in the form of a cut-off index (COI; signal sample/cut-off).	< 1.0 Negative, ≥ 1.0 Positive
Abbott Architect SARS-CoV-2	Chemiluminescent microparticle immunoassay	Nucleocapsid	30	IgG	The resulting chemiluminescent reaction is measured as a relative light unit (RLU).	RLU (Relative Light Intensity)	One Positive calibrator	Results are reported by dividing the sample result by the calibrator result (mean of 3 calibrators). The default result unit for the SARS-CoV-2 IgG assay is Index (S/C).	< 1.4 Negative, ≥ 1.4 Positive
IT3 AbC-19	Rapid Point of Care Lateral Flow Immunoassay	Full length Spike protein	20	IgG	The colour intensity of the test line is analysed using the reference score card.	Binary	The presence of a control line indicates the test is valid.	A result is positive if there is both a test line and a control line, whilst a result is negative if only the control line is present.	Using the reference score card; Positive scores ≥1 Negative scores=0

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

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

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

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

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

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

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

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

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

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

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

Supplementary Methods

Laboratory-based immunoassays

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

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

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

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

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

Analytical specificity and sensitivity assessment

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

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5-6
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
Participants	**6**	Eligibility criteria	7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6/7
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6/7
	9	Whether participants formed a consecutive, random or convenience series	6
Test methods	**10a**	Index test, in sufficient detail to allow replication	8
	10b	Reference standard, in sufficient detail to allow replication	8
	11	Rationale for choosing the reference standard (if alternatives exist)	5-6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	8, supp table 1
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Supp methods, supp table 1
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Supp methods
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8/9
	15	How indeterminate index test or reference standard results were handled	11, Supp Fig1
	16	How missing data on the index test and reference standard were handled	Supp Fig 1
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	12-14
	18	Intended sample size and how it was determined	8-9
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Supp Fig 1
	20	Baseline demographic and clinical characteristics of participants	10
	21a	Distribution of severity of disease in those with the target condition	10
	21b	Distribution of alternative diagnoses in those without the target condition	10
	22	Time interval and any clinical interventions between index test and reference standard	6-7
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Fig 3, Fig S3, S5-S7
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	12-13, Table 1
	25	Any adverse events from performing the index test or the reference standard	n/a
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	4, 18-20
	27	Implications for practice, including the intended use and clinical role of the index test	19-20
OTHER INFORMATION			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents

	30	Sources of funding and other support; role of funders	22

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Northern Ireland nationwide serological study for laboratory evaluation of SARS-CoV-2 antibodies: detectable IgG more than 10 months post infection.

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Northern Ireland nationwide serological study for laboratory evaluation of SARS-CoV-2 antibodies: detectable IgG more than 10 months post infection.

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Abstract

Objective

To evaluate the dynamics and longevity of the humoral immune response to SARS-CoV-2 infection and assess the performance of professional use of the UK-RTC AbC-19 Rapid Test lateral flow immunoassay (LFIA) for the target condition of SARS-CoV-2 spike protein IgG antibodies.

Design

Nationwide serological study.

Setting

Northern Ireland, UK, May 2020- February 2021.

Participants

Plasma samples were collected from a diverse cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations and research studies (n=223) and through a convalescent plasma program (n=183). Plasma donors (n=101) were followed with sequential samples over 11 months post symptom onset.

Main Outcome Measures

SARS-CoV-2 antibody levels in plasma samples using Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2 ELISA immunoassays over time. UK-RTC AbC-19 LFIA sensitivity and specificity, estimated using a three-reference standard system to establish a characterised panel of 330 positive and 488 negative SARS-CoV-2 IgG samples.

Results

We detected persistence of SARS-CoV-2 IgG antibodies for up to 10 months post infection, across a minimum of two laboratory immunoassays. On the known positive cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58% (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-99.95%).

Conclusions

Through comprehensive analysis of a cohort of pre-pandemic and pandemic individuals, we show detectable levels of IgG antibodies, lasting over 46 weeks when assessed by EuroImmun ELISA, providing insight to antibody levels at later time points post-infection. We show good laboratory validation performance metrics for the AbC-

19 rapid test for SARS-CoV-2 spike protein IgG antibody detection in a laboratory-based setting.

Strengths and Limitations

- Strength - This paper describes a non-clinical laboratory evaluation and comparison of the ability of three different immunoassays to detect SARS-CoV-2 antibodies in the same samples, detecting different subtypes of antibodies against different targets of the viral antigenic repertoire, that does not rely on PCR-positivity as definition of expected test outcome, to provide a panel of known antibody positive and antibody negative serology for evaluation of newly developed immunoassays.

- Strength - This study demonstrates AbC-19 lateral flow point of care detection of IgG antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the antibodies made in response to the vaccines used globally, in a large cohort of subjects, more than 10 months post infection, across a broad age range (18-78 years).

- Strength - This study assesses correlation between approved laboratory-based assays and the AbC-19 lateral flow point of care lateral flow test for the detection of SARS-CoV-2 antibodies in characterised cohorts of known positive and negative plasma samples in an evaluation conducted according to MHRA guidelines during a pandemic.

- Strength- Longitudinal data detecting IgG antibodies more than 10 months from infection was collected as sequential samples over time through a convalescent plasma donation program.

- Limitation- This study was conducted in a standardised setting with very experienced users on plasma characterised as positive or negative for the presence of antibodies using a reference standard, alongside one other assay which may introduce a possible spectrum bias and may not reflect the true performance metrics of the assay evaluated when translated to real life settings, using finger prick blood samples and in which pre-test probability would impact greatly on positive and negative predictive values.

Keywords

SARS-CoV-2, COVID-19, immunoassay, LFIA, pandemic, antibody assay

Introduction

The World Health Organization declared a pandemic in March 2020 due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), identified late 2019 in Wuhan, China, causing Coronavirus Disease 2019 (COVID-19) disease (1,2).

A global race ensued to develop diagnostic assays, with the most common being viral RNA detection (RT-qPCR assays), to detect acute infection(3). RT-qPCR assays are labour and reagent intensive, limited by a short temporal window for positive diagnosis, and exhibit potential for false negative results (4). Evidence suggests sensitivity of RT-qPCR can be as low as 70% (5). False positive rates between 0.8- 4.0% have been reported in the UK and are dependent on the Ct values accepted as indicating

infection, the number of SARS-CoV-2 genes analysed, and the proportion of asymptomatic individuals tested (6,7). Lockdown measures and "flattening the curve" strategies in the UK meant many infected individuals were instructed to self-isolate and were not offered a diagnostic RT-qPCR, with much of the testing limited to patients admitted to hospital, who perhaps reflect a more severely infected cohort. Consequently, a potentially large number of cases were unconfirmed or undetected (8).

The ability to accurately detect SARS-CoV-2 specific antibodies, which develop after an immune response is evoked, is vital for building biobanks of convalescent sera for treatment, monitoring immune response to infection alongside surveillance studies and assessing responses to vaccination programmes.

Commercial serology immunoassays are mostly laboratory-based and measure IgG antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIAs), require a finger prick blood sample and can be used at point-of-care (POC) or in the home; particularly important in the context of lockdown enforcement during the pandemic. A limited number of laboratory-based chemiluminescence immunoassays are approved for use in the UK including the Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region (Roche Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay against the same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

The complexities of the humoral immune response to SARS-CoV-2 is a much-debated topic. In a US study, approximately one in 16 individuals lacked detectable IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR confirmed infection (9). Patients who remain asymptomatic may mount a humoral immune response which is short-lived, with detectable levels of antibody falling

rapidly (10). This, alongside the lack of RT-PCR test availability across the UK has hindered development of well characterised gold standard serology test for IgG antibodies to SARS-CoV-2.

Herein, we describe the use of Roche and Abbott commercial immunoassays, as well as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike (antigenic) protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-pandemic and pandemic COVID-19 blood samples (n=880) from within Northern Ireland and report on longevity of IgG antibodies detected. Furthermore, we follow IgG antibody levels in convalescent plasma donors (n=101 individuals) for up to 11 months. Currently, there is no gold standard assay for comparison, therefore we aimed to establish a reference based on a positive COVID-19 antibody status. We present results of a laboratory evaluation of the UK-RTC AbC-19 with a target condition of antibodies against a cohort of 330 known IgG antibody positive samples according to this 'positive by two' system and 488 negative samples (223 pre-pandemic assumed negative and 265 known negative) for IgG to SARS-CoV-2.

Methods

Participant samples

The flow of participant samples is summarised in Figure S1. A small cohort (n=19) of anonymised plasma samples were obtained from a partner USA laboratory for initial protocol development only. All participants provided informed consent. An online recruitment strategy was employed, with the study advertised through internal Ulster University email, website and social media. A BBC Newsline feature providing the pandemic study email address also prompted interest from the general population.

The first 800 respondents who expressed interest were provided with an online patient information sheet, consent form and health questionnaire and invited to register to attend a clinic. Participants were eligible for the study if they were over 18 years of age. Exclusion criteria included anyone with a blood disorder or contraindication to giving a blood sample, or anyone currently exhibiting symptoms of COVID-19. To enrich the cohort for samples potentially positive for SARS-CoV-2 IgG antibody, further participants were invited if they had previously tested PCR positive or had the distinctive symptom of loss of taste and smell. Blood sampling clinics were held at locations around Northern Ireland between May and July 2020 resulting in collection of 263 10ml EDTA plasma samples from 263 separate study participants. Additional anonymised plasma samples were obtained from Southern Health and Social Care Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood Transfusion Service (NIBTS, n=184) through convalescent plasma programs. NIBTS convalescent plasma samples continued to be collected throughout 2020-early 2021, with a total of n=897 from n=676 individuals, including n=183 samples from the cross-sectional cohort. Individuals from this program with a positive RT-PCR result and EuroImmun starting value >6 were sequentially sampled over a period of up to 46 weeks resulting in a cohort of n=101 individuals, n=296 samples (including n=47 individuals from the cross-sectional cohort).

Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster University ethics committee approved studies with ongoing consent and from NIBTS (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-thaw cycles for all analyses reported within this manuscript.

Clinical information

Basic demographic information and data with regard to probable or definite prior infection with SARS-CoV-2 was obtained from PANDEMIC study participants through the secure online questionnaire requiring responses about positive RT-PCR result and/or time from symptom onset. Anonymised participant samples from USA, SHSCT and NIBTS were provided with age, gender and time since PCR-positive, where a previous test had been carried out.

Laboratory-based immunoassays

Details of laboratory immunoassays are summarised in supplementary methods and Table S1.

UK-RTC AbC-19 LFIA

All analyses were performed on UK-RTC AbC-19 Technical Transfer 3 (TT3) devices at Ulster University according to manufacturer's instructions (details in Table S1). Assays were performed as cohorts, with samples in batches of 10, with one researcher adding 2.5µL of plasma to the assay and a second adding 100µL of buffer immediately following sample addition. After 20 minutes, the strength of each resulting test line was scored from 0-10 according to a visual score card (scored by 3 researchers; Figure S2). A score ≥1 was positive. Details of samples used for analysis for detection of antibodies are available in Supplementary methods.

Statistical analysis

As per Daniel (11) a minimum sample size based on prevalence can be calculated using the following formula: $n = \frac{Z^2 P(1-P)}{d^2}$, where n = sample size, Z = Z statistic for a

chosen level of confidence, P = estimated prevalence, and d = precision. Assuming a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the required sample size at 99% confidence (Z = 2.58) to be 240 individuals. If the true prevalence is lower, 5%, the estimated required sample size given a precision of 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody immunoassays(12).

Statistical analysis was conducted in in R v 4.0.2(13). To assess discordance between test results, data was first filtered to include individuals with an Abbott test result in the range ≥0.25 & ≤1.4, with a 2 x 2 contingency table produced that comprised all possible combinations of [concordant|discordant] test results [within|outside of] this range. A p-value was derived via a Pearson χ^2 test after 2000 p-value simulations via the stats package.

AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To compare test result (Positive|Negative) to age, a binary logistic regression model was produced with test result as outcome – a p-value was then derived via χ^2 ANOVA. To compare time against test result (encoded continuously), a linear regression was performed. We calculated median per time-period and then converted these to log [base 2] ratios against the positivity cut-off for each assay. All plots were generated via ggplot2 or custom functions using base R(14).

Results

We analysed samples from a mixed cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations

and research studies (n=223) and through a convalescent plasma program (n=183). Antibody levels in plasma from these 880 individuals were assessed using the three SARS-CoV-2 immunoassays; EuroImmun IgG, Roche Elecsys IgG/IgM/IgA and Abbott Architect IgG (Table S1, Figure S3). This included a cohort of 223 pre-pandemic plasma samples collected and stored during 2017 to end of May 2019 to determine assay specificity. Of the 657 participants whose samples were collected during the pandemic, 267 (40.64%) previously tested RT-PCR positive with a range of 7-173 days since diagnosis. A total of 225 participants gave time since self-reported COVID-19 symptoms, with a range of 5-233 days from symptom onset, whilst 195 had no symptom or PCR data available. Samples collected in 2020 (n=657) ranged from 19-78 years of age with a median (IQR) of 43 years (±22), and n=454 were female and n=200 male (n=3, not disclosed). Pre-pandemic samples (n=223) ranged from 20-87 years of age with median (IQR) of 50 years (±20) and consisted of n=88 female and n=135 male.

Laboratory based antibody immunoassays

A positive result for antibody on one or more of the three laboratory immunoassays was recorded for 385/657 (58.6%) participants who provided a sample during the pandemic. By EuroImmun ELISA, 346 were positive, 20 borderline and 291 were negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott determined 310 positive and 347 negative (Table S2, Figure S3). The median age across all age groups combined was lower for participants testing positive across each of the immunoassays (median [sd] for positive versus negative, respectively: EuroImmun, 41 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41 [13.18] vs 47 [13.09]). (Figure S4, $p<0.0001$). When segregated by age group, however, differences were less apparent in certain groups (Figure S5). Excluding the

pre-pandemic cohort, this gap reduced but remained statistically significant EuroImmun, 41 [13.18] vs 45 [12.49]; Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41 [13.26] vs 44 [12.63]) ($p<0.01$) (median [sd] for positive versus negative). Of note, out of 267 individuals with a previous positive RT-PCR result for SARS-CoV-2 viral RNA, 14 (5.2%, Figure S3a) did not show detectable antibodies by any of the three immunoassays, with no association found with age, gender or time between test and blood draw (data not shown).

The three commercial laboratory immunoassays provide a ratio value that increases with IgG antibody titre. When correlation between these values is assessed, good overall agreement is observed between the three immunoassays (Figure 1, Figure S5). As highlighted by Rosadas *et al*., we also see significant disagreement in the Abbott 0.25-1.4 range when compared to EuroImmun and Roche (Figure 1a,b; chi-square p-values: EuroImmun vs Abbott, $p<0.001$; Roche vs Abbott, $p<0.001$)(15).

Duration of humoral response to SARS-CoV-2

In a cross-sectional analysis of antibodies over time, we found IgG antibodies could still be detected in individuals (excluding pre-pandemic) across all three immunoassays used up to week 20 (day 140) (Figure 2). We note a statistically significant decrease in signal with respect to time across each assay (p-value [estimate slope]): EuroImmun, $p=0.028$[-0.823]; Roche, $p=0.002$ [-0.125]; Abbott, $p<0.0001$ [-3.673]. These remained statistically significant after adjustment for age. Antibody levels (expressed as a ratio of median result per timepoint divided by positivity cut off; Table 1) peaked at Week 1-2 for EuroImmun (1.33) and Abbott (1.64), though reached highest levels at Week 8-12 when measured by Roche (5.45). By week 21-24, median score for all tests had dropped below the positivity

cut off, though a small number of samples remained above the positive cut off at these later timepoints (Figure 2).

Samples from the NIBTS convalescent plasma program continued to be collected throughout 2020-early 2021. A total of n= 897 samples from n=676 individuals were collected, 744/883 tested by EuroImmun were positive (>1.1, with values range of 0.051-34.361), 556/749 tested by Abbott were positive (>1.4, with values ranging from 0.01-8.85). Individuals with a positive RT-PCR result and a EuroImmun result >6 were sequentially sampled (with median 3, range 2-9 samples per individual) and analysed by both EuroImmun (n=101 individuals), and Abbott immunoassays (n=75 individuals). Median age (IQR) for this cohort is 51 years (±21) with a range from 18-70 years and n=27 female, n=74 male. Longitudinal analysis shows persistence of detectable IgG antibodies until up to 302 days (43 weeks) by Abbott immunoassay (at which point this assay was discontinued at NIBTS) and 323 days (46 weeks) by EuroImmun ELISA, with a gradual decline over time (Figure S6). None of the individuals who were initially positive by Euroimmun SARS-CoV-2 S1 IgG assay dropped to below the EuroImmun positivity threshold (>1.1) over the course of the follow-up while 26 who were initially positive by Abbott SARS-CoV-2 NP IgG fell below the Abbott threshold (>1.4).

UK-RTC AbC-19

Using the commercial immunoassays described we established a well characterised serology sample set of ‘known positive’ and ‘known negative’ for IgG antibodies to SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.

AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore required all samples to be positive by the EuroImmun SARS-CoV-2 IgG ELISA, which

likewise detects antibodies against the S1 domain (16). To develop this characterised cohort, samples were also required to be positive by a second immunoassay (Roche or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG antibody, we assessed 350 plasma samples from participants classed as 'known negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from individuals confirmed to be negative across all three laboratory assays (Roche, EuroImmun, Abbott). Using these positive n=304 and negative n=350 antibody cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the AbC-19 LFIA (Table 2).

Given a recent report of lower specificity in the AbC-19 LFIA (17) and the possibility of introducing sample bias, we revised our inclusion criteria for the negative cohort. For the pre-pandemic cohort, we included samples from all 223 individuals, regardless of results on other laboratory immunoassays. When this assumed negative pre-pandemic cohort was used for laboratory evaluation for target condition of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 2). We expanded the negative cohort to include all samples that matched our criteria (samples collected during the pandemic to be negative by all three laboratory assays and all pre-pandemic samples regardless of other immunoassay results). The specificity observed on this extended negative cohort of 488 samples was 99.59% (98.53% to 99.95%, Table 2). For sensitivity analysis on a positive cohort (samples positive by EuroImmun and one other test), we were able to analyse all samples previously untested due to limited testing capacity and tested a positive cohort of 330 samples giving a sensitivity of 97.58% (95.28% to 98.95%, Table 2). When we sorted samples analysed in both negative (n=488) and positive cohorts (n=330) by

RT-PCR status and assessed AbC-19 LFIA sensitivity by including only those that were RT-PCR positive (n=227), the test showed a sensitivity of 92.07% (87.76%-95.23%, Table S3). However, of the n=18 RT-PCR positive individuals negative for IgG antibodies by AbC-19, n=12 showed no detectable antibodies by all three laboratory assays (EuroImmun, Roche or Abbott), suggesting that antibodies are not present in those samples.

When used as intended by the public, the AbC-19 LFIA provides binary positive/negative results. However, when assessing LFIA in the laboratory, each test line was scored against a scorecard by three independent researchers (0 negative, 1-10 positive; Figure S2). When compared to quantitative outputs from the Abbott, EuroImmun and Roche assays, the AbC-19 LFIA shows good correlation (Abbott r=0.84 [p<0.001]; EuroImmun r=0.86 [p<0.001]; Roche r=0.82 [p<0.001]; Figure 3, Figure S7-Figure S9).

Analytical specificity and sensitivity of AbC-19 LFIA

We observed no cross-reactivity across samples with known H5N1 influenza, Respiratory syncytial virus, Influenza A, Influenza B, Bordetella Pertussis, Haemophilus Influenzae, Seasonal coronavirus NL63 and 229E on the AbC-19 LFIA (n=34 samples, n=8 distinct respiratory viruses; Table S4). Against a panel of external reference SARS-CoV-2 serology samples, the AbC-19 LFIA detected antibodies with scores commensurate to the EuroImmun ELISA scores (Figure S10, Table S5).

Discussion

Serological antibody immunoassays are an important tool in helping combat the SARS-CoV-2 pandemic. The duration of the humoral immune response is of particular importance, to inform an individual's protection following both natural infection and vaccination. Using a large cohort of individuals across a wide age range (18-78), we assessed antibody levels across up to three laboratory immunoassays perform a cross-sectional and longitudinal analysis over time. Our results show strong correlation between all three immunoassays, with shortcomings in the Abbott system output 0.25-1.4 range, as described previously, suggesting an overestimated positive cut-off (Figure 1) (15).

Longitudinal studies on SARS-CoV-1 convalescent patients suggests that detectable IgG can still be present as long as 2 years after infection (18). There are conflicting reports of the longevity of the humoral response to SARS-CoV-2 infection which differ in the make-up of the cohort studied, the assays used, and the length of time since symptom onset. The longevity of IgG antibodies to both spike and nucleocapsid protein more than 10 months after RT PCR positive status (and beyond in a small number of samples, Figure 2, Figure S6) is consistent with that observed in other recent studies(19–21). In this study, samples were collected through a convalescent plasma program (Figure S6), with individuals selected for sequential plasma donation based on an initial high EuroImmun assay score. In contrast to the time series analysis of healthcare workers recruited prospectively by Manisty *et al*., we observed no cases where Euroimmun ELISA-measured anti-Spike antibody levels fell below threshold, whilst a large number of Abbott measured anti-Nucleocapsid antibody levels dropped below the positivity threshold (34.7% 26/75). However, this may be an overestimate given the shortcomings of the Abbott assay

described above (Figure 1) (22). In a similar longitudinal study of 51 symptomatic participants, Dan *et al*. estimated that half-life ($t_{1/2}$) for IgG-Spike (103 days) was longer than that for IgG-Nucleocapsid (68 days), although with a considerable overlap of 95% confidence intervals (23).

In our more diverse cross-sectional cohort, we also note a statistically significant decline over time but levels remain detectable at 140 days (Figure 2). We note that IgG levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as Week 8-12 from first symptoms or a viral RNA RT-PCR positive result, though this may be an artefact of lower number of participants at earlier timepoints (Table 1). Robust antibody responses are produced in our cohorts across a wide age range (18-78 years old, Figure 2, Figure S6). We detect a slightly but significantly lower median age of participants testing positive (Figure S4); however, this is likely be due to cohort characteristics and not a true reflection of the population or indication of test performance.

A difficulty faced in validation of antibody diagnostic assays has been access to samples with known SARS-CoV-2 antibody status. As previously described, there is no clear gold standard reference against which to assess SARS-CoV-2 immunoassays. A positive RT-PCR test has been used previously to indicate previous (COVID-19) SARS-CoV-2 infection, though this approach is limited by a high rate of false negatives and positives in RT-PCR testing, failure in some cases to develop IgG antibodies (sero-silence or lack of antibody against the same antigenic component of the virus as the immunoassay uses as a capture antigen) and the lack of RT-PCR testing availability early in the pandemic (3,5,24). SARS-CoV-2 IgG antibodies were undetectable in 14 of 267 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA

positive participants in this study. It is unclear if this is due to insufficient/absent antibody production in these individuals at the time the sample was taken, or due to a false positive PCR result which may occur in the UK at a rate between 0.8- 4.0% (6). Self-assessment of symptoms for COVID-19 (disease) is a poor indicator of previous infection, even amongst healthcare workers (25). Additionally, the kinetics of a SARS-CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus with time, contributing to false negative RT-PCR test results for individuals who may be late to present for virus detection tests (5,26).

To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-CoV-2 antibody in a laboratory evaluation, we developed a reference standard for SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar approach was used in a recent seroprevalence study in Iceland, whereby two positive antibody results were required to determine a participant sample as positive for SARS-CoV-2 antibody (24). Our evaluation of performance metrics for the UK-RTC AbC-19 LFIA to detect antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59% specificity. In an evaluation of the AbC-19 tests, Mulchandani *et al*. observed a specificity of 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report a sensitivity of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a previous RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys antibody test, which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid portion of SARS-CoV-2 (25). In RT-PCR positive individuals from our cohorts, the AbC-19 test showed a similar sensitivity (92.07%, 87.76%- 95.23%, Table S3). However, we demonstrate the drawbacks of this approach given that in 12 of 18 AbC-19 false negatives, none of the four immunoassays used (EuroImmun, Roche, Abbott

or AbC-19) detected antibodies, suggesting either a false RT-PCR result, a failure to produce IgG antibodies or sero-reversion before sample collection in these individuals. Another recent evaluation of the AbC-19 LFIA by Moshe *et al*. determined a sensitivity of (100% (98.1-100%) on laboratory sera, using a composite reference standard of antibody positive by either Spike protein ELISA or hybrid DABA assay and specificity of 99.8% (98.9-100%) against pre-pandemic samples. However, when AbC-19 performance was analysed on matched finger-prick and serum samples against the same antibody standard, a lower sensitivity was observed (finger-prick 69% (53.8-81.3%), serum 92% (80-97.7%)) (27).

In our study, strong correlation was observed in quantitative score between results on all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA (Figure S8, S9). This is to be expected, given both the AbC-19 LFIA and EuroImmun ELISA detect IgG antibodies against spike protein. Importantly, for the assessment of immunity to prior natural infection as well as to immunisation, IgG antibodies against SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA and AbC-19 LFIA are known to correlate with neutralizing antibodies, which may confer future immunity (23,28,29). Previous evaluations of sensitivity and specificity reported by Public Health England (PHE), showed a EuroImmun sensitivity of 72% and specificity of 99%, Abbott with sensitivity of 92.7% and specificity of 100% and Roche with sensitivity of 83.9% and specificity of 100% (30–32). The PHE analyses for each of these tests used previous infection (RT-PCR positive status) as a reference standard, the limitations of which are discussed above.

In the use of characterised 'known positive' and 'known negative' cohorts, one limitation of this study is its potential for spectrum bias, whereby our positive-by-two reference system may artificially raise the threshold for positive sample inclusion, possibly resulting in the overestimation of the sensitivity of any test evaluated (33). However, similar issues have been raised when using previous RT-PCR result or definitive COVID-19 symptoms as inclusion criteria given these will likely skew a cohort towards more severe disease, especially given issues of RT-PCR availability outside of hospital settings during the first wave (5). Importantly, our mixed origin of samples forming the cohort provides a positive cohort for assessing assay sensitivity that includes individuals from the general public, healthcare workers and from convalescent plasma programmes. In the absence of a clear gold standard test, our system relies on no single test (each with their individual shortcomings) and instead takes an average of three. Our analysis of specificity on only pre-pandemic individuals (n=223) shows similar specificity (99.55%) to the larger mixed 'known negative cohort' (n=488, sensitivity 99.59%). We also demonstrate a high level of analytical specificity of the AbC-19 test with no cross-reactivity against a panel of other respiratory viruses, including SARS-CoV-1 NL63 and 229E (Table S4).

Our assessment of the AbC-19 LFIA in a laboratory setting, using characterised cohorts of known SARS-CoV-2 antibody positive and antibody negative plasma, shows good performance metrics for its ability to detect SARS-CoV-2 IgG antibodies following natural infection. We note our use of plasma from venous blood samples, as opposed to a finger prick blood sample as would be used in rapid testing scenarios (27). Additionally, when the AbC-19 LFIA was used on our cohort, a number of the positive results scored low (1/10 using the score card under laboratory conditions,

Figure 3), with a faint test band visible to a trained laboratory scientist but perhaps difficult to identify as positive by individuals performing a single test (Figure S10). This faint line may be reflective of the longer time from infection for the Northern Ireland cohort used. If the AbC-19 LFIA is to be used in clinical settings it is important to determine if all users observe the same results as observed in this laboratory evaluation.

This assessment of the AbC-19 LFIA does not provide data on how this test will perform in a seroprevalence screening scenario, but instead provides metrics for the performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as opposed to previous COVID-19 infection. An important potential use of the AbC-19 LFIA would be in monitoring the immune response to vaccination, with most vaccines utilising SARS-CoV-2 Spike protein antigens (34).

Conclusion

We present a comprehensive analysis of pre-pandemic and two large pandemic cohorts (more than 700 individuals) and in a longitudinal analysis showing that IgG antibodies to SARS-CoV-2 antigens are detectable more than 10 months from positive RT-PCR test. We use antibody positive status as an alternative to RT-PCR positive status as a standard for assessing SARS-CoV-2 antibody assays and show strong performance for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-CoV-2 antibodies. User experience in future studies in the real world is important and may alter the performance characteristics. Also, the effect of operator training will have direct effects upon test performance. We welcome further clinical evaluation of the AbC-19 LFIA in large cohorts of symptomatic and asymptomatic individuals alongside

large studies assessing vaccination outcomes in individuals to fully validate its implementation across all intended use cases.

Declarations

Ethics approval and consent to participate

All study participants provided informed consent. This study was approved by Ulster University Institutional Ethics committee (REC/20/0043), South Birmingham REC (The PANDEMIC Study IRAS Project ID: 286041Ref 20/WM/0184) and adhered to the Declaration of Helsinki and Good Clinical Practice.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Consent for publication

Not applicable.

Dissemination to participants and related patient and public communities.

Links to this work will be included on the study website (https://www.ulster.ac.uk/coronavirus/research/research-output/pandemic-study) and participants will be alerted that the work has been published.

Data sharing

Data are available on reasonable request to the corresponding author.

Competing interests:

At the time of this study TM and JML acted as advisors to CIGA HealthCare, an industrial partner in the UK Rapid Test Consortium. No personal financial reward or renumeration was received for this advisory role. At the time of submission of this manuscript TM and JML no longer held these advisory positions.

All other authors have no potential conflict of interest to report.

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The advisory roles within CIGA Healthcare were unpaid temporary roles. This manuscript and associated data within this paper has only been used to build confidence into the overall device design and performance assessment of the UK RTC AbC-19 devices and such work was never commissioned for any government contractual consideration.

Authors' contributions:

TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR, SM and KYN analysed data, KB performed all statistical analyses/interpretations and produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and provided Blood Transfusion cohort samples. TM, RP and AN coordinated participant recruitment, consent and sampling. WB and JML developed online consent forms, questionnaires and databases. LR, JM, AK, AA, GEW, DH, SS, CCS performed sample collection and processing. LR and TM wrote the manuscript, with significant contributions from JM, AN and KB. All authors reviewed and approved the final manuscript.

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Table 1: Antibody level ratios for assays over time

Antibody level ratios for assays over time show varying peak levels depending on test. Calculated by first establishing the median per time period, then calculating log2 ratio for each period versus each respective assay positivity cut-off.

	Ratio median antibody level: assay positivity cut-off									
	Week									
	Pre-2020	1-2	3-4	5-8	9-12	13-16	18-20	21-24	25-28	29+
EuroImmun	-2.65	1.33	0.2	0.95	1.32	0.47	0.04	-2.01	-2.26	-2.01
Roche	-3.64	3.16	3.05	5.20	5.45	4.14	4.42	-3.54	-3.69	-3.61
Abbott	-5.54	1.64	-0.51	1.12	0.86	0.08	-0.59	-5.13	-5.13	-6.13
Sample number (n=)	223	20	10	52	90	202	53	11	12	11

Table 2: UK-RTC AbC-19 LFIA performance metrics against known antibody positive and known antibody negative cohorts.

Total Negative	True Negative	False Positive	Total Positive	True Positive	False Negative	Sensitivity % (95 CI)	Specificity % (95 CI)
Pre-pandemic (n=223)							
223	222	1	n/a	n/a	n/a	n/a	99.55% (97.53% to 99.99%)
Initially reported cohorts (n=654)							
350	350	0	304	297	7	97.70% (95.31%-99.07%)	100.00% (98.95%-100.00%)
Extended cohorts (n=818)							
488	486	2	330	322	8	97.58% (95.28%-98.95%)	99.59% (98.53%-99.95%)

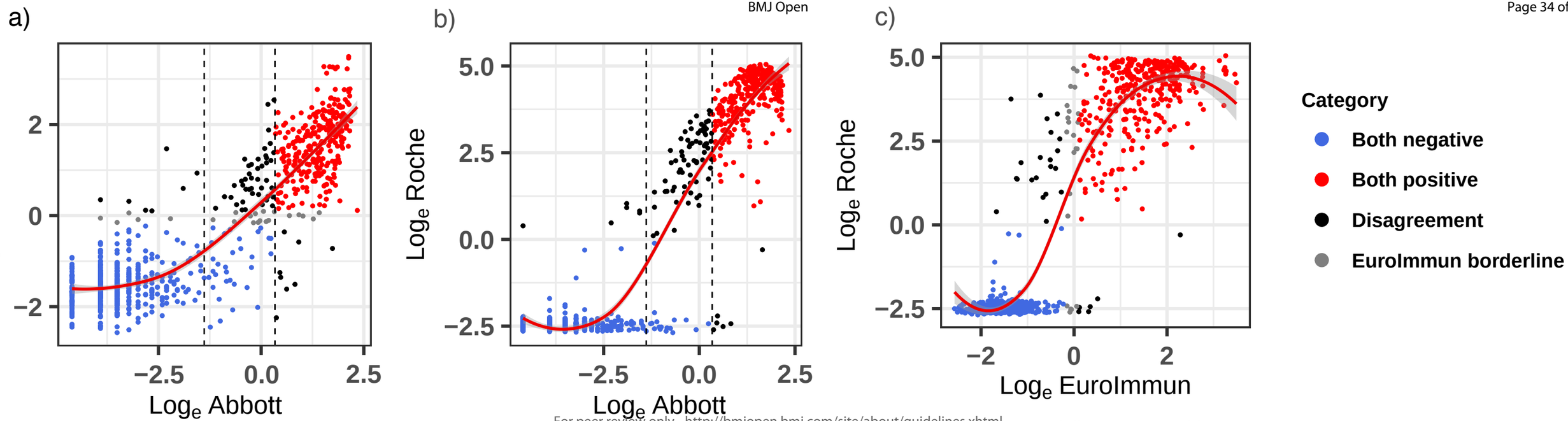
Figure Legends

Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott and c) Roche immunoassays. Pearson χ^2 test was used to assess correlations. The results for each test were log transformed to ensure results follow a normal distribution. Negative agreement shown as blue dots, red dots show positive agreement for the two immunoassays, whilst black dots show disagreement and grey dots as the EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4. n=880. The graphs show positive correlations between all immunoassays evaluated, with the fewest disagreement of results between the Log of Roche and the Log of EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.

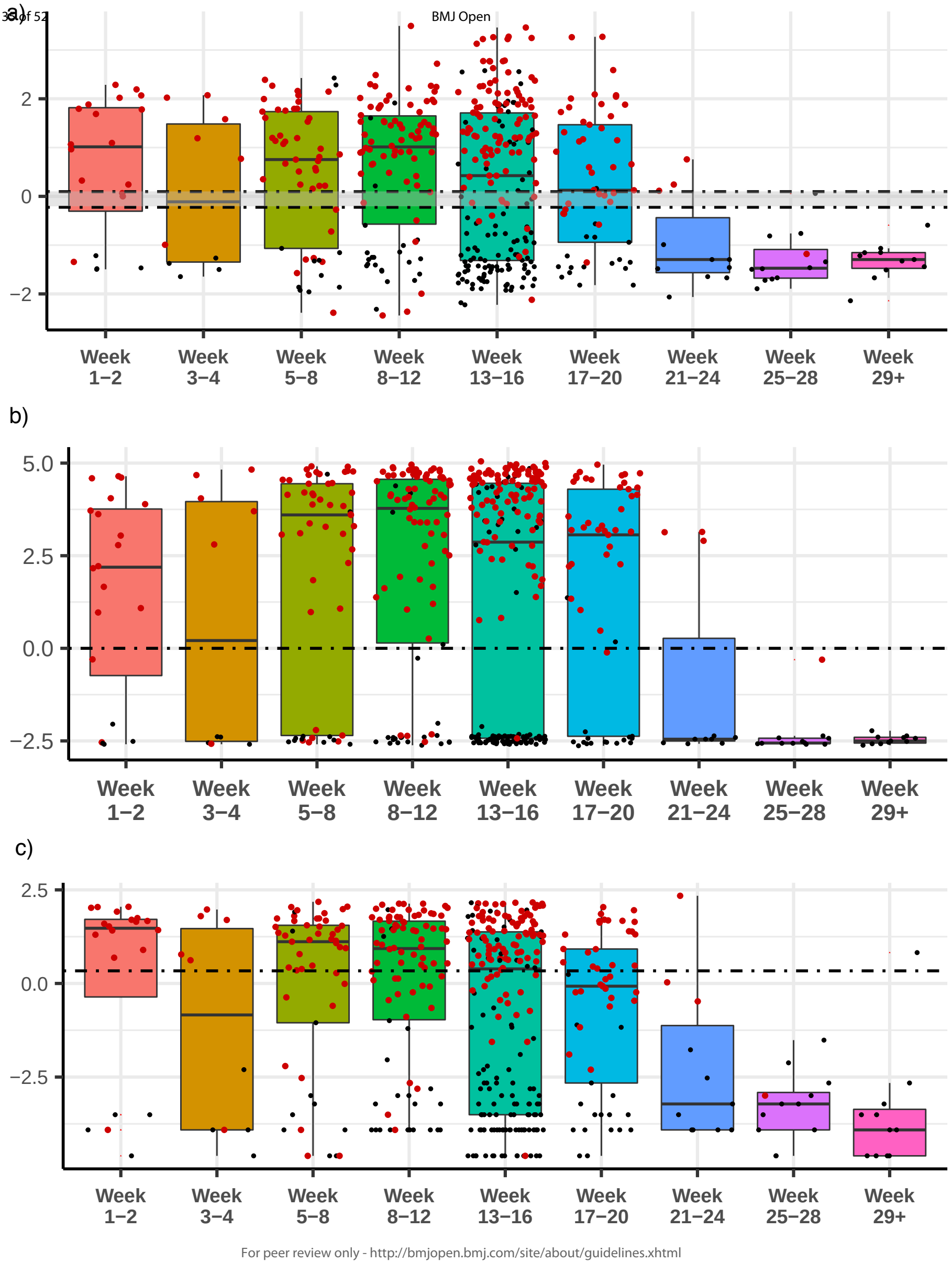
Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c) Abbott, relative to weeks since first reported symptoms or positive PCR result (where data available, n=685). RT-PCR positive individuals are denoted by red dots, while individuals with time since symptom data are denoted in black. Dashed lines delineate $\log_e$ equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result between the two lines). Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).

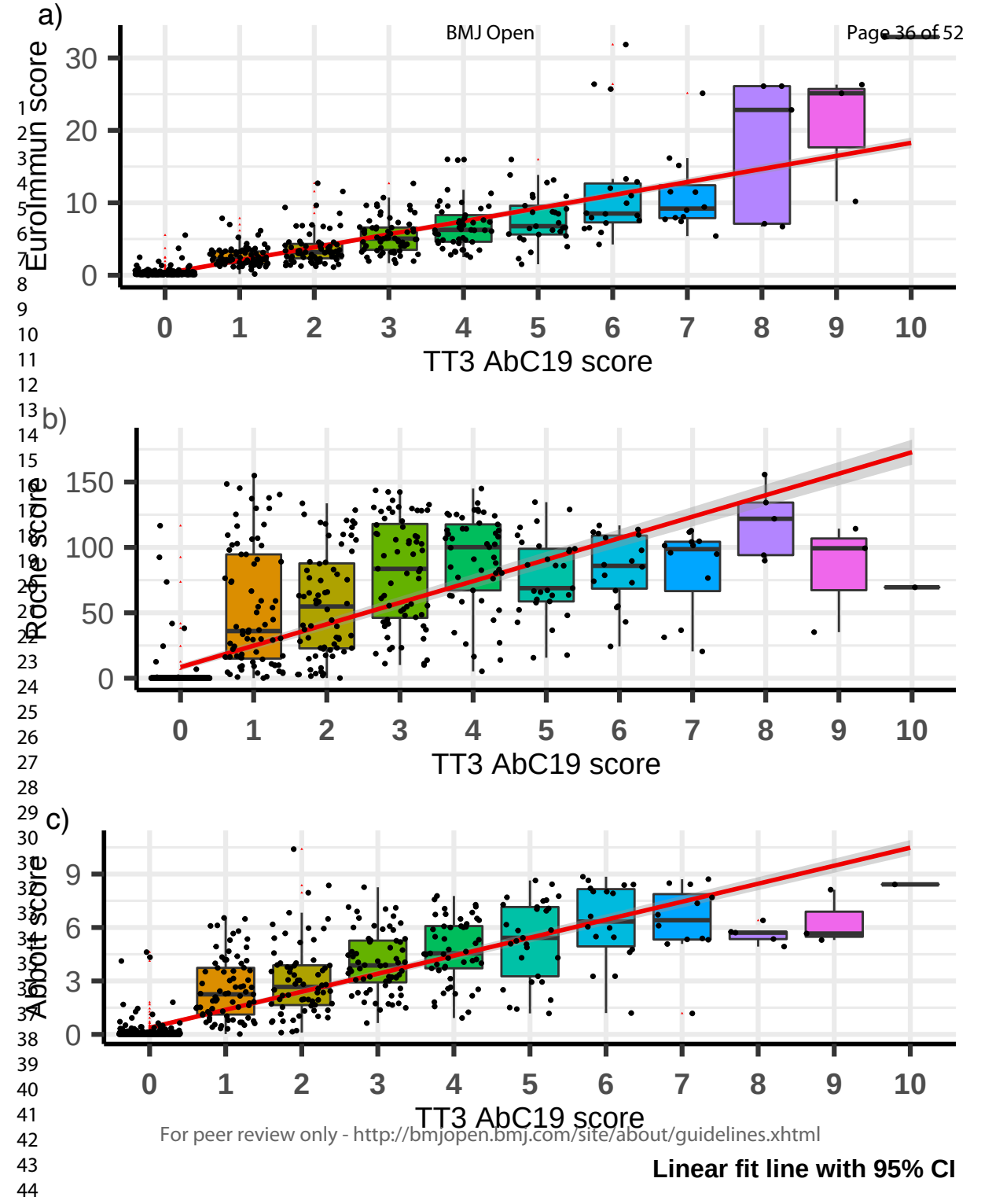
Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing AbC-19 TT3 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line

of best fit with 95% confidence interval shaded in grey. Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).



Fit lines, LOESS with 95% CI
Vertical lines mark Abbott test range 0.25−1.4


a)
Log$_e$ EuroImmun
2
0
−2
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+
b)
Log$_e$ Roche
5.0
2.5
0.0
−2.5
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+
c)
Log$_e$ Abbott
2.5
0.0
−2.5
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+



Linear fit line with 95% CI

Supplementary Materials

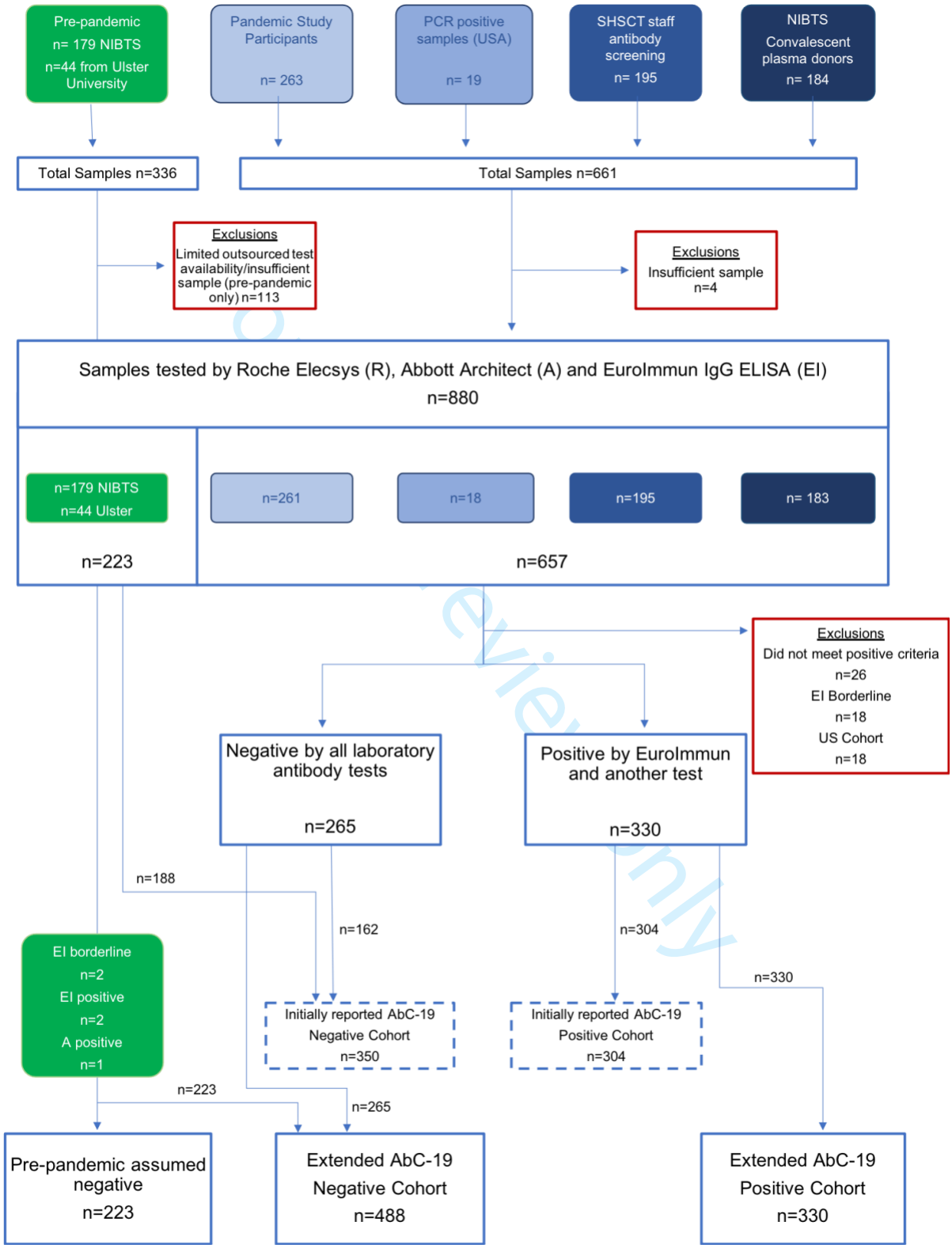
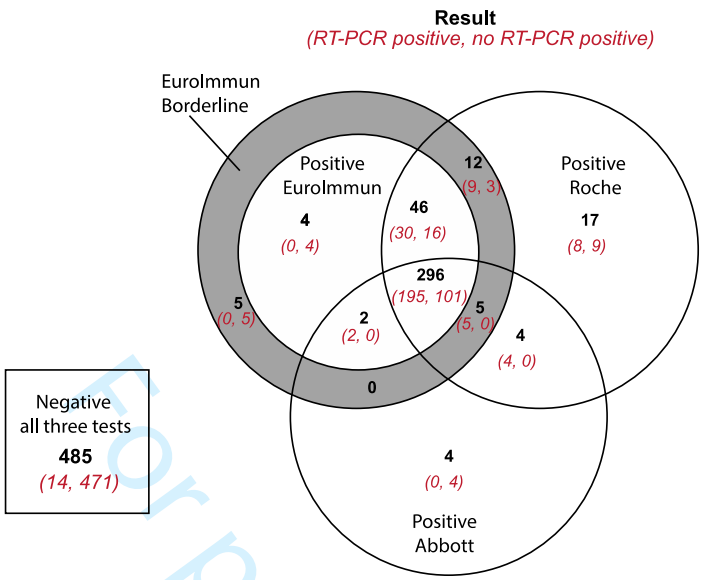


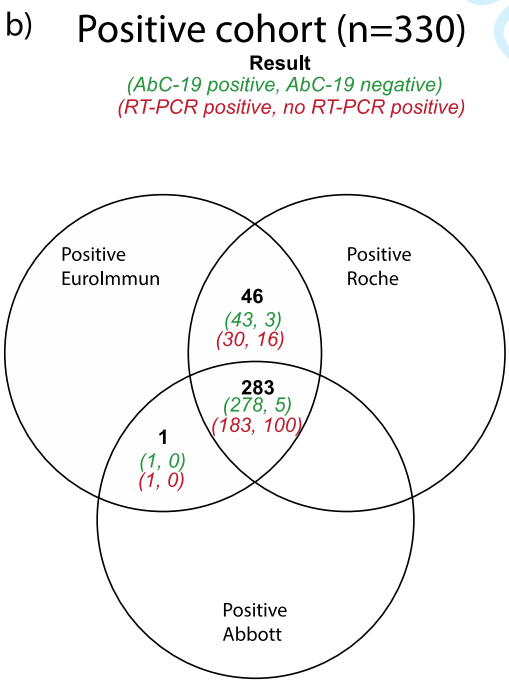
Figure S1: Flow of participant plasma samples through cross-sectional study. All available samples from participants within each cohort, and the included and excluded samples at all stages. Freeze thaw cycles were closely monitored for all sample aliquots. Pre-pandemic samples taken forward for Roche, Abbott and EuroImmun testing were selected based on aliquot volume and availability.

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

a)



b)



c)

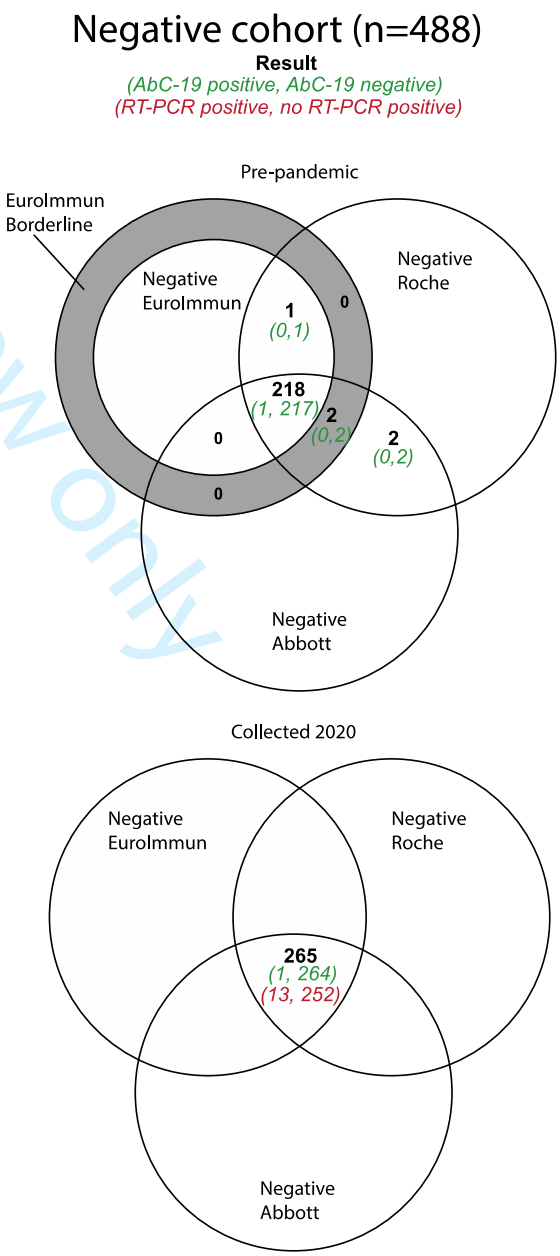


Figure S3: Venn diagrams demonstrating result overlap between laboratory assays in a) the initial immunoassay cohort (n=880), b) the positive and c) negative cohorts assessed with AbC-19 TT3. Result in each circle overlap in bold, (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below.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

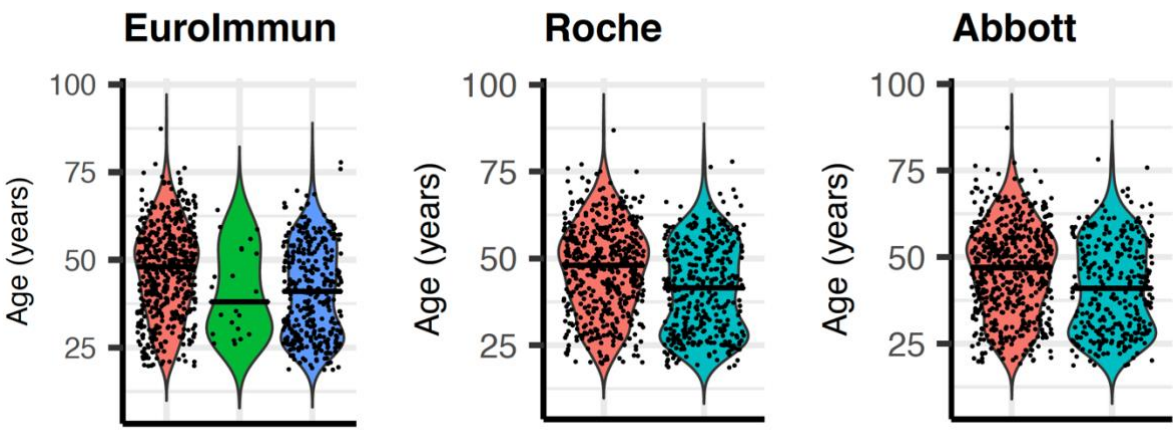


Figure S4: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.

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

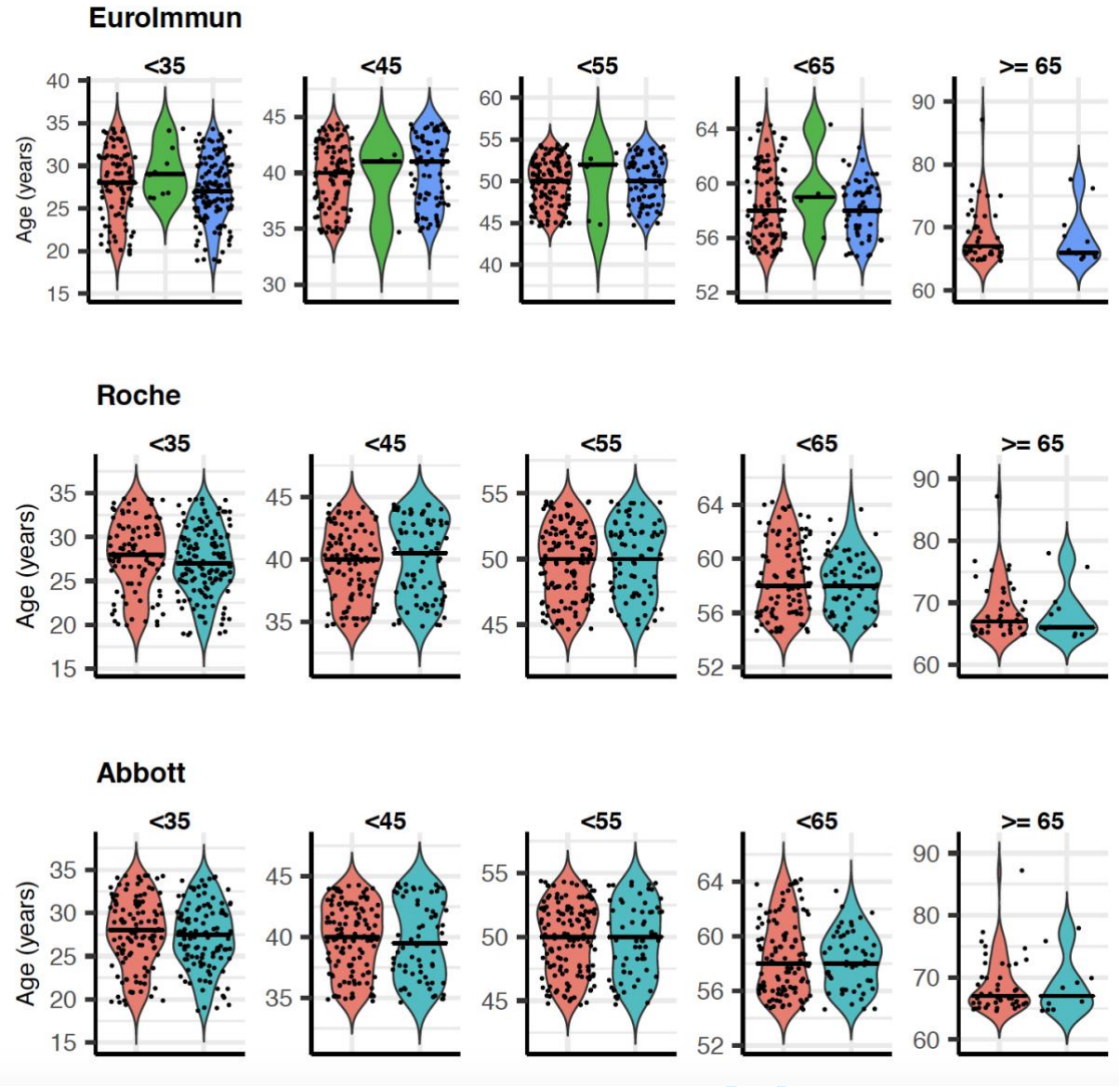


Figure S5: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.

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

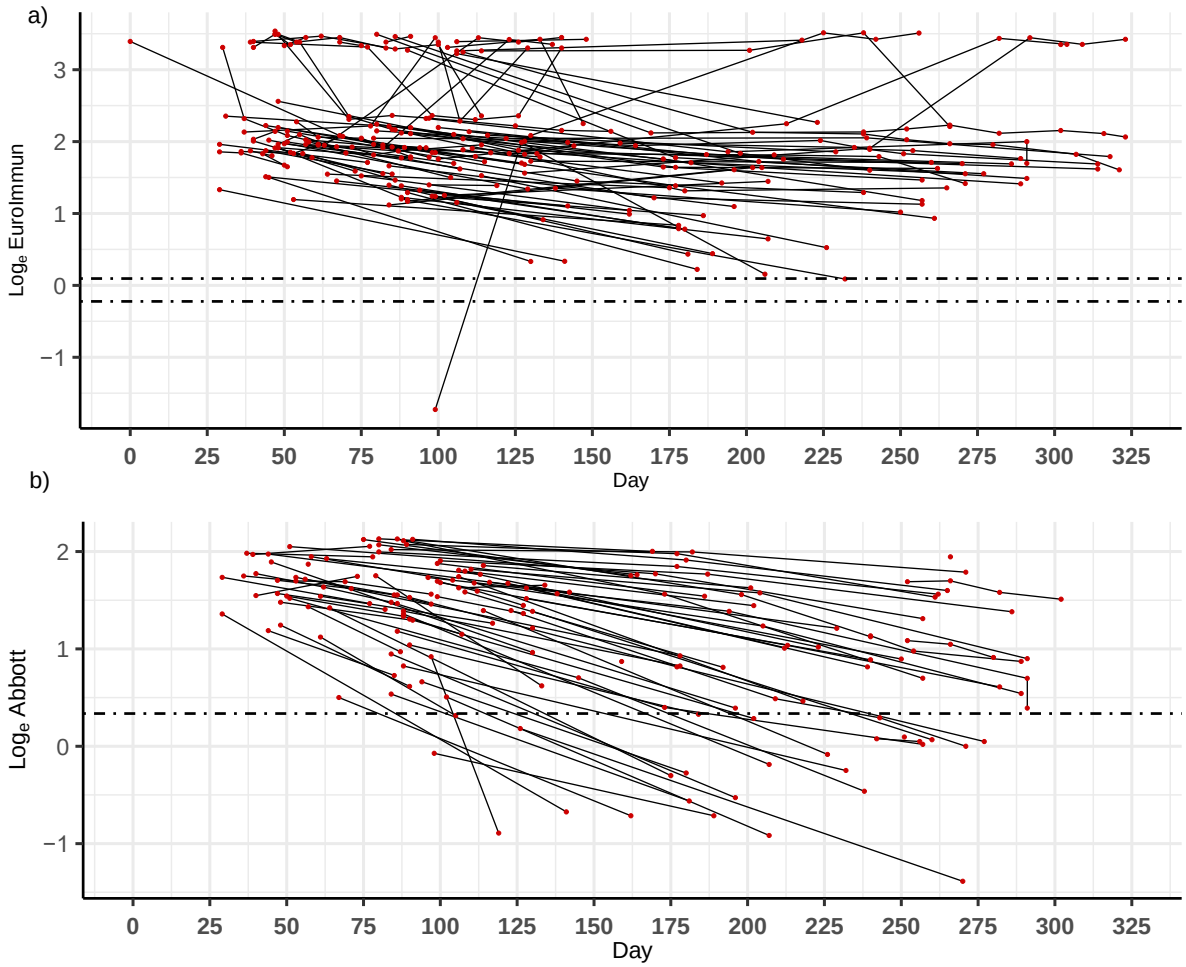


Figure S6: Longitudinal analysis of convalescent plasma donor sequential samples (2-9 samples per individual) by a) EuroImmun ELISA or b) Abbott immunoassay. a) n=101 individuals, grey shading indicates borderline region, upper dotted line indicates positivity threshold (1.1), lower dotted line indicates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

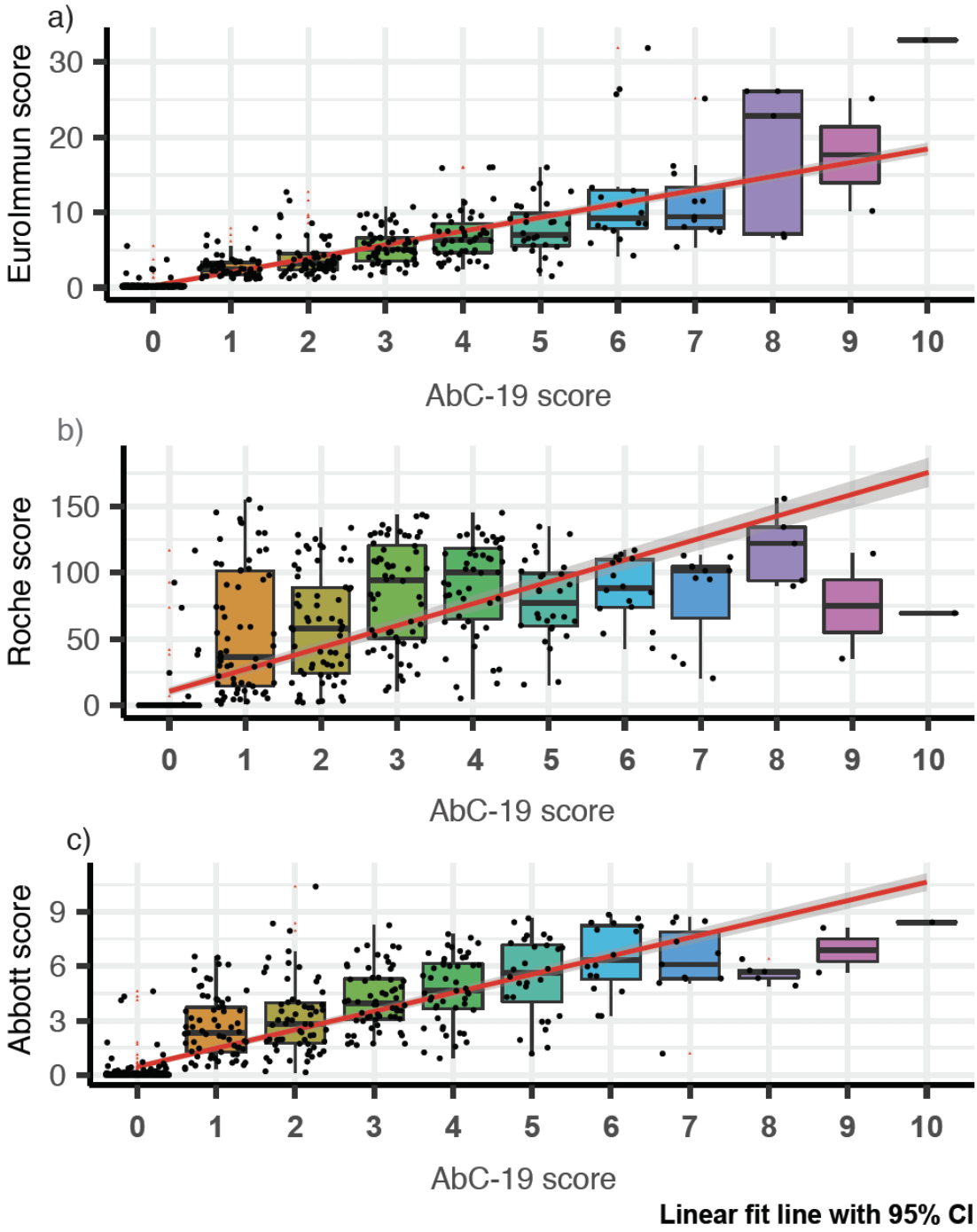


Figure S7: AbC-19 initially reported cohort n=654 correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing TT3 AbC-19 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line of best fit with 95% confidence interval shaded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

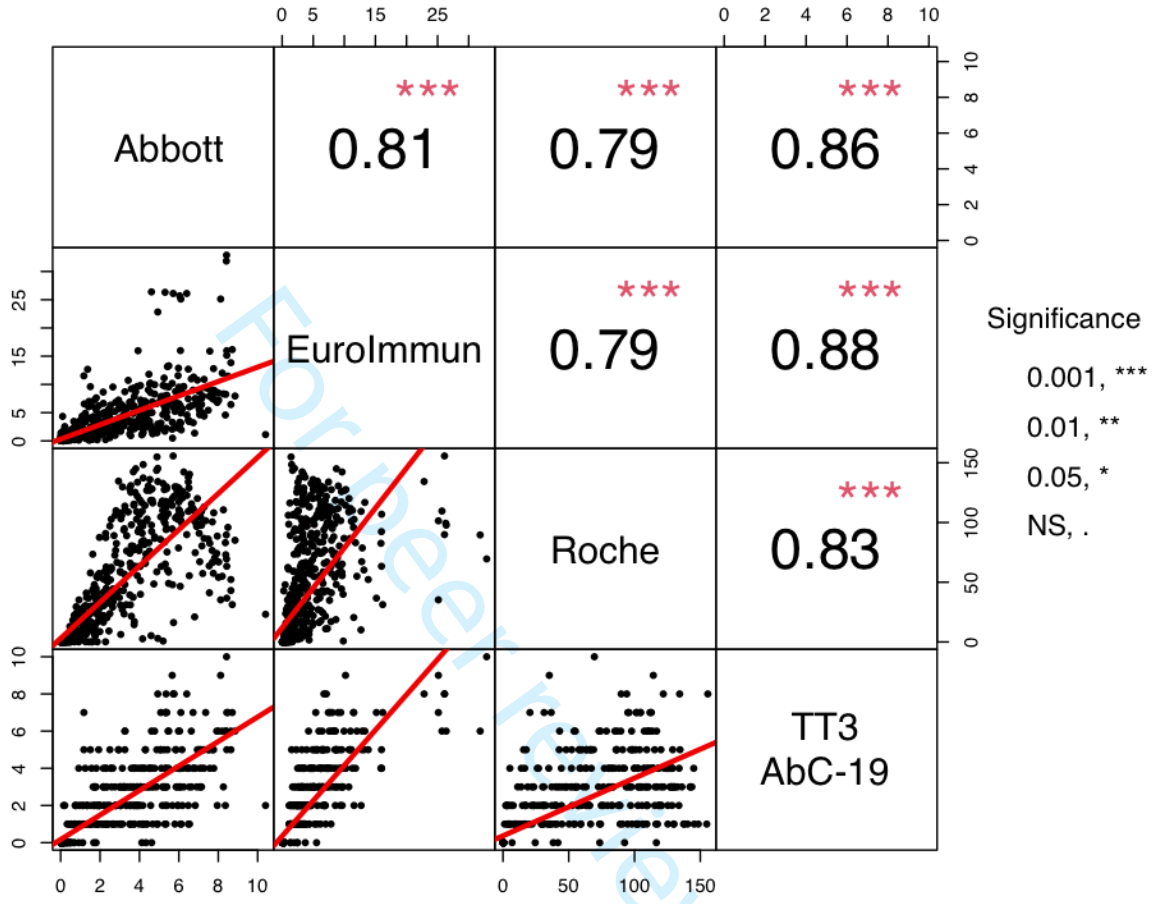


Figure S8: **Correlation matrix between Abbott, EuroImmun, Roche and initially reported AbC-19 cohort (n=654) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly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

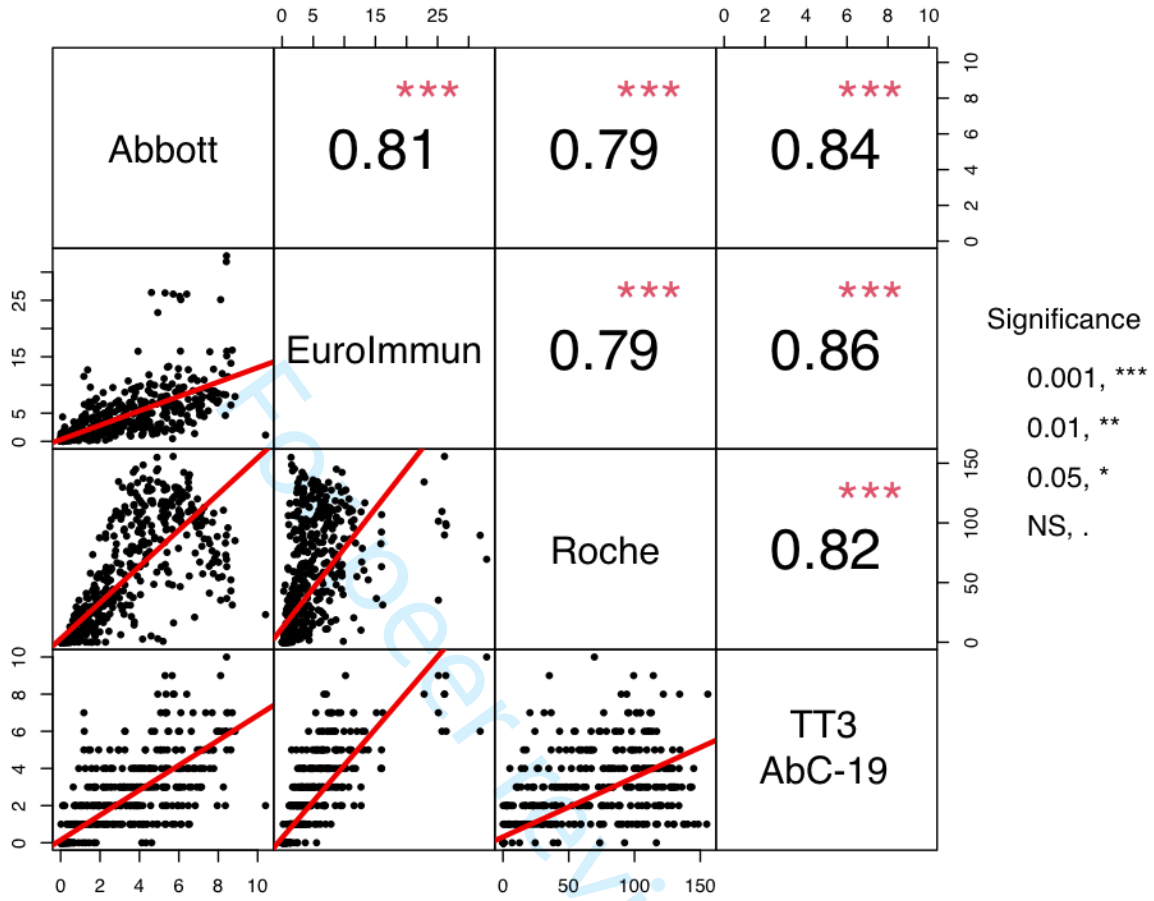


Figure S9: **Correlation matrix between Abbott, EuroImmun, Roche and extended AbC-19 cohort (n=818) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly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

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

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

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

Immunoassay	Principle	Antigen Target	Assay Time (min)	Antibody Detected	Measurement	Result	Calibration	Evaluation of results	Results
EuroImmun ELISA	Enzyme-linked immunosorbent assay (enzyme-HRP)	S1 domain of the spike protein	120	IgG	Photometric measurement of the color intensity using wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm	OD (Optical density)	One Positive calibrator	OD of clinical sample/OD of calibrator	< 0.8 Negative, ≥ 0.8 to <1.1 Borderline, ≥ 1.1 Positive
Roche Elecsys immunoassay	Electro-chemiluminescence	Nucleocapsid	18	IgG, IgA and IgM	Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier	RLU (Relative Light Intensity)	One Positive calibrator and one Negative calibrator	The analyzer automatically calculates the cut-off based on the measurement of ACOV2 Cal1 (negative) and ACOV2 Cal2 (positive). The result of a sample is given either as reactive or non-reactive as well as in the form of a cut-off index (COI; signal sample/cut-off).	< 1.0 Negative, ≥ 1.0 Positive
Abbott Architect SARS-CoV-2	Chemiluminescent microparticle immunoassay	Nucleocapsid	30	IgG	The resulting chemiluminescent reaction is measured as a relative light unit (RLU).	RLU (Relative Light Intensity)	One Positive calibrator	Results are reported by dividing the sample result by the calibrator result (mean of 3 calibrators). The default result unit for the SARS-CoV-2 IgG assay is Index (S/C).	< 1.4 Negative, ≥ 1.4 Positive
IT3 AbC-19	Rapid Point of Care Lateral Flow Immunoassay	Full length Spike protein	20	IgG	The colour intensity of the test line is analysed using the reference score card.	Binary	The presence of a control line indicates the test is valid.	A result is positive if there is both a test line and a control line, whilst a result is negative if only the control line is present.	Using the reference score card; Positive scores ≥1 Negative scores=0

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

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

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

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

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

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

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

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

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

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

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

Supplementary Methods

Laboratory-based immunoassays

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

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

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

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

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

Analytical specificity and sensitivity assessment

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

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5-6
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
Participants	**6**	Eligibility criteria	7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6/7
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6/7
	9	Whether participants formed a consecutive, random or convenience series	6
Test methods	**10a**	Index test, in sufficient detail to allow replication	8
	10b	Reference standard, in sufficient detail to allow replication	8
	11	Rationale for choosing the reference standard (if alternatives exist)	5-6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	8, supp table 1
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Supp methods, supp table 1
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Supp methods
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8/9
	15	How indeterminate index test or reference standard results were handled	11, Supp Fig1
	16	How missing data on the index test and reference standard were handled	Supp Fig 1
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	12-14
	18	Intended sample size and how it was determined	8-9
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Supp Fig 1
	20	Baseline demographic and clinical characteristics of participants	10
	21a	Distribution of severity of disease in those with the target condition	10
	21b	Distribution of alternative diagnoses in those without the target condition	10
	22	Time interval and any clinical interventions between index test and reference standard	6-7
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Fig 3, Fig S3, S5-S7
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	12-13, Table 1
	25	Any adverse events from performing the index test or the reference standard	n/a
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	4, 18-20
	27	Implications for practice, including the intended use and clinical role of the index test	19-20
OTHER INFORMATION			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents

	30	Sources of funding and other support; role of funders	22

BMJ Open

Evaluation of the IgG antibody response to SARS CoV-2 infection and performance of a lateral flow immunoassay: cross-sectional and longitudinal analysis over 11 months

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	Nanotechnology and Integrated Bioengineering Centre Moore, Tara; Ulster University, Biomedical Sciences Research Institute
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	COVID-19, Molecular diagnostics < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES

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Evaluation of the IgG antibody response to SARS CoV-2 infection and performance of a lateral flow immunoassay: cross-sectional and longitudinal analysis over 11 months

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Abstract

Objective

To evaluate the dynamics and longevity of the humoral immune response to SARS-CoV-2 infection and assess the performance of professional use of the UK-RTC AbC-19 Rapid Test lateral flow immunoassay (LFIA) for the target condition of SARS-CoV-2 spike protein IgG antibodies.

Design

Nationwide serological study.

Setting

Northern Ireland, UK, May 2020- February 2021.

Participants

Plasma samples were collected from a diverse cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations and research studies (n=223) and through a convalescent plasma program (n=183). Plasma donors (n=101) were followed with sequential samples over 11 months post symptom onset.

Main Outcome Measures

SARS-CoV-2 antibody levels in plasma samples using Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2 ELISA immunoassays over time. UK-RTC AbC-19 LFIA sensitivity and specificity, estimated using a three-reference standard system to establish a characterised panel of 330 positive and 488 negative SARS-CoV-2 IgG samples.

Results

We detected persistence of SARS-CoV-2 IgG antibodies for up to 10 months post infection, across a minimum of two laboratory immunoassays. On the known positive cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58% (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-99.95%).

Conclusions

Through comprehensive analysis of a cohort of pre-pandemic and pandemic individuals, we show detectable levels of IgG antibodies, lasting over 46 weeks when assessed by EuroImmun ELISA, providing insight to antibody levels at later time points post-infection. We show good laboratory validation performance metrics for the AbC-

19 rapid test for SARS-CoV-2 spike protein IgG antibody detection in a laboratory-based setting.

Strengths and Limitations

- Strength - This paper describes a non-clinical laboratory evaluation and comparison of the ability of three different immunoassays to detect SARS-CoV-2 antibodies in the same samples, detecting different subtypes of antibodies against different targets of the viral antigenic repertoire, that does not rely on PCR-positivity as definition of expected test outcome, to provide a panel of known antibody positive and antibody negative serology for evaluation of newly developed immunoassays.

- Strength - This study demonstrates AbC-19 lateral flow point of care detection of IgG antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the antibodies made in response to the vaccines used globally, in a large cohort of subjects, more than 10 months post infection, across a broad age range (18-78 years).

- Strength - This study assesses correlation between approved laboratory-based assays and the AbC-19 lateral flow point of care lateral flow test for the detection of SARS-CoV-2 antibodies in characterised cohorts of known positive and negative plasma samples in an evaluation conducted according to MHRA guidelines during a pandemic.

- Strength- Longitudinal data detecting IgG antibodies more than 10 months from infection was collected as sequential samples over time through a convalescent plasma donation program.

- Limitation- This study was conducted in a standardised setting with very experienced users on plasma characterised as positive or negative for the presence of antibodies using a reference standard, alongside one other assay which may introduce a possible spectrum bias and may not reflect the true performance metrics of the assay evaluated when translated to real life settings, using finger prick blood samples and in which pre-test probability would impact greatly on positive and negative predictive values.

Keywords

SARS-CoV-2, COVID-19, immunoassay, LFIA, pandemic, antibody assay

Introduction

The World Health Organization declared a pandemic in March 2020 due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), identified late 2019 in Wuhan, China, causing Coronavirus Disease 2019 (COVID-19) disease (1,2).

A global race ensued to develop diagnostic assays, with the most common being viral RNA detection (RT-qPCR assays), to detect acute infection(3). RT-qPCR assays are labour and reagent intensive, limited by a short temporal window for positive diagnosis, and exhibit potential for false negative results (4). Evidence suggests sensitivity of RT-qPCR can be as low as 70% (5). False positive rates between 0.8- 4.0% have been reported in the UK and are dependent on the Ct values accepted as indicating

infection, the number of SARS-CoV-2 genes analysed, and the proportion of asymptomatic individuals tested (6,7). Lockdown measures and "flattening the curve" strategies in the UK meant many infected individuals were instructed to self-isolate and were not offered a diagnostic RT-qPCR, with much of the testing limited to patients admitted to hospital, who perhaps reflect a more severely infected cohort. Consequently, a potentially large number of cases were unconfirmed or undetected (8).

The ability to accurately detect SARS-CoV-2 specific antibodies, which develop after an immune response is evoked, is vital for building biobanks of convalescent sera for treatment, monitoring immune response to infection alongside surveillance studies and assessing responses to vaccination programmes.

Commercial serology immunoassays are mostly laboratory-based and measure IgG antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIAs), require a finger prick blood sample and can be used at point-of-care (POC) or in the home; particularly important in the context of lockdown enforcement during the pandemic. A limited number of laboratory-based chemiluminescence immunoassays are approved for use in the UK including the Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region (Roche Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay against the same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

The complexities of the humoral immune response to SARS-CoV-2 is a much-debated topic. In a US study, approximately one in 16 individuals lacked detectable IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR confirmed infection (9). Patients who remain asymptomatic may mount a humoral immune response which is short-lived, with detectable levels of antibody falling

rapidly (10). This, alongside the lack of RT-PCR test availability across the UK has hindered development of well characterised gold standard serology test for IgG antibodies to SARS-CoV-2.

Herein, we describe the use of Roche and Abbott commercial immunoassays, as well as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike (antigenic) protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-pandemic and pandemic COVID-19 blood samples (n=880) from within Northern Ireland and report on longevity of IgG antibodies detected. Furthermore, we follow IgG antibody levels in convalescent plasma donors (n=101 individuals) for up to 11 months. Currently, there is no gold standard assay for comparison, therefore we aimed to establish a reference based on a positive COVID-19 antibody status. We present results of a laboratory evaluation of the UK-RTC AbC-19 with a target condition of antibodies against a cohort of 330 known IgG antibody positive samples according to this 'positive by two' system and 488 negative samples (223 pre-pandemic assumed negative and 265 known negative) for IgG to SARS-CoV-2.

Methods

Participant samples

The flow of participant samples is summarised in Figure S1. A small cohort (n=19) of anonymised plasma samples were obtained from a partner USA laboratory for initial protocol development only. All participants provided informed consent. An online recruitment strategy was employed, with the study advertised through internal Ulster University email, website and social media. A BBC Newsline feature providing the pandemic study email address also prompted interest from the general population.

The first 800 respondents who expressed interest were provided with an online patient information sheet, consent form and health questionnaire and invited to register to attend a clinic. Participants were eligible for the study if they were over 18 years of age. Exclusion criteria included anyone with a blood disorder or contraindication to giving a blood sample, or anyone currently exhibiting symptoms of COVID-19. To enrich the cohort for samples potentially positive for SARS-CoV-2 IgG antibody, further participants were invited if they had previously tested PCR positive or had the distinctive symptom of loss of taste and smell. Blood sampling clinics were held at locations around Northern Ireland between May and July 2020 resulting in collection of 263 10ml EDTA plasma samples from 263 separate study participants. Additional anonymised plasma samples were obtained from Southern Health and Social Care Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood Transfusion Service (NIBTS, n=184) through convalescent plasma programs. NIBTS convalescent plasma samples continued to be collected throughout 2020-early 2021, with a total of n=897 from n=676 individuals, including n=183 samples from the cross-sectional cohort. Individuals from this program with a positive RT-PCR result and EuroImmun starting value >6 were sequentially sampled over a period of up to 46 weeks resulting in a cohort of n=101 individuals, n=296 samples (including n=47 individuals from the cross-sectional cohort).

Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster University ethics committee approved studies with ongoing consent and from NIBTS (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-thaw cycles for all analyses reported within this manuscript.

Clinical information

Basic demographic information and data with regard to probable or definite prior infection with SARS-CoV-2 was obtained from PANDEMIC study participants through the secure online questionnaire requiring responses about positive RT-PCR result and/or time from symptom onset. Anonymised participant samples from USA, SHSCT and NIBTS were provided with age, gender and time since PCR-positive, where a previous test had been carried out.

Laboratory-based immunoassays

Details of laboratory immunoassays are summarised in supplementary methods and Table S1.

UK-RTC AbC-19 LFIA

All analyses were performed on UK-RTC AbC-19 Technical Transfer 3 (TT3) devices at Ulster University according to manufacturer's instructions (details in Table S1). Assays were performed as cohorts, with samples in batches of 10, with one researcher adding 2.5µL of plasma to the assay and a second adding 100µL of buffer immediately following sample addition. After 20 minutes, the strength of each resulting test line was scored from 0-10 according to a visual score card (scored by 3 researchers; Figure S2). A score ≥1 was positive. Details of samples used for analysis for detection of antibodies are available in Supplementary methods.

Statistical analysis

As per Daniel (11) a minimum sample size based on prevalence can be calculated using the following formula: $n = \frac{Z^2 P(1-P)}{d^2}$, where n = sample size, Z = Z statistic for a

chosen level of confidence, P = estimated prevalence, and d = precision. Assuming a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the required sample size at 99% confidence (Z = 2.58) to be 240 individuals. If the true prevalence is lower, 5%, the estimated required sample size given a precision of 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody immunoassays(12).

Statistical analysis was conducted in in R v 4.0.2(13). To assess discordance between test results, data was first filtered to include individuals with an Abbott test result in the range ≥0.25 & ≤1.4, with a 2 x 2 contingency table produced that comprised all possible combinations of [concordant|discordant] test results [within|outside of] this range. A p-value was derived via a Pearson χ^2 test after 2000 p-value simulations via the stats package.

AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To compare test result (Positive|Negative) to age, a binary logistic regression model was produced with test result as outcome – a p-value was then derived via χ^2 ANOVA. To compare time against test result (encoded continuously), a linear regression was performed. We calculated median per time-period and then converted these to log [base 2] ratios against the positivity cut-off for each assay. All plots were generated via ggplot2 or custom functions using base R(14).

Results

We analysed samples from a mixed cohort of individuals from the general public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations

and research studies (n=223) and through a convalescent plasma program (n=183). Antibody levels in plasma from these 880 individuals were assessed using the three SARS-CoV-2 immunoassays; EuroImmun IgG, Roche Elecsys IgG/IgM/IgA and Abbott Architect IgG (Table S1, Figure S3). This included a cohort of 223 pre-pandemic plasma samples collected and stored during 2017 to end of May 2019 to determine assay specificity. Of the 657 participants whose samples were collected during the pandemic, 267 (40.64%) previously tested RT-PCR positive with a range of 7-173 days since diagnosis. A total of 225 participants gave time since self-reported COVID-19 symptoms, with a range of 5-233 days from symptom onset, whilst 195 had no symptom or PCR data available. Samples collected in 2020 (n=657) ranged from 19-78 years of age with a median (IQR) of 43 years (±22), and n=454 were female and n=200 male (n=3, not disclosed). Pre-pandemic samples (n=223) ranged from 20-87 years of age with median (IQR) of 50 years (±20) and consisted of n=88 female and n=135 male.

Laboratory based antibody immunoassays

A positive result for antibody on one or more of the three laboratory immunoassays was recorded for 385/657 (58.6%) participants who provided a sample during the pandemic. By EuroImmun ELISA, 346 were positive, 20 borderline and 291 were negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott determined 310 positive and 347 negative (Table S2, Figure S3). The median age across all age groups combined was lower for participants testing positive across each of the immunoassays (median [sd] for positive versus negative, respectively: EuroImmun, 41 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41 [13.18] vs 47 [13.09]). (Figure S4, $p<0.0001$). When segregated by age group, however, differences were less apparent in certain groups (Figure S5). Excluding the

pre-pandemic cohort, this gap reduced but remained statistically significant EuroImmun, 41 [13.18] vs 45 [12.49]; Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41 [13.26] vs 44 [12.63]) ($p<0.01$) (median [sd] for positive versus negative). Of note, out of 267 individuals with a previous positive RT-PCR result for SARS-CoV-2 viral RNA, 14 (5.2%, Figure S3a) did not show detectable antibodies by any of the three immunoassays, with no association found with age, gender or time between test and blood draw (data not shown).

The three commercial laboratory immunoassays provide a ratio value that increases with IgG antibody titre. When correlation between these values is assessed, good overall agreement is observed between the three immunoassays (Figure 1a-c, Figure S5). As highlighted by Rosadas *et al*., we also see significant disagreement in the Abbott 0.25-1.4 range when compared to EuroImmun and Roche (Figure 1a,b; chi-square p-values: EuroImmun vs Abbott, $p<0.001$; Roche vs Abbott, $p<0.001$)(15).

Duration of humoral response to SARS-CoV-2

In a cross-sectional analysis of antibodies over time, we found IgG antibodies could still be detected in individuals (excluding pre-pandemic) across all three immunoassays used up to week 20 (day 140) (Figure 2). We note a statistically significant decrease in signal with respect to time across each assay (p-value [estimate slope]): EuroImmun, $p=0.028$[-0.823]; Roche, $p=0.002$ [-0.125]; Abbott, $p<0.0001$ [-3.673]. These remained statistically significant after adjustment for age. Antibody levels (expressed as a ratio of median result per timepoint divided by positivity cut off; Table 1) peaked at Week 1-2 for EuroImmun (1.33) and Abbott (1.64), though reached highest levels at Week 8-12 when measured by Roche (5.45). By week 21-24, median score for all tests had dropped below the positivity

cut off, though a small number of samples remained above the positive cut off at these later timepoints (Figure 2).

Samples from the NIBTS convalescent plasma program continued to be collected throughout 2020-early 2021. A total of n= 897 samples from n=676 individuals were collected, 744/883 tested by EuroImmun were positive (>1.1, with values range of 0.051-34.361), 556/749 tested by Abbott were positive (>1.4, with values ranging from 0.01-8.85). Individuals with a positive RT-PCR result and a EuroImmun result >6 were sequentially sampled (with median 3, range 2-9 samples per individual) and analysed by both EuroImmun (n=101 individuals), and Abbott immunoassays (n=75 individuals). Median age (IQR) for this cohort is 51 years (±21) with a range from 18-70 years and n=27 female, n=74 male. Longitudinal analysis shows persistence of detectable IgG antibodies until up to 302 days (43 weeks) by Abbott immunoassay (at which point this assay was discontinued at NIBTS) and 323 days (46 weeks) by EuroImmun ELISA, with a gradual decline over time (Figure S6). None of the individuals who were initially positive by Euroimmun SARS-CoV-2 S1 IgG assay dropped to below the EuroImmun positivity threshold (>1.1) over the course of the follow-up while 26 who were initially positive by Abbott SARS-CoV-2 NP IgG fell below the Abbott threshold (>1.4).

UK-RTC AbC-19

Using the commercial immunoassays described we established a well characterised serology sample set of ‘known positive’ and ‘known negative’ for IgG antibodies to SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.

AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore required all samples to be positive by the EuroImmun SARS-CoV-2 IgG ELISA, which

likewise detects antibodies against the S1 domain (16). To develop this characterised cohort, samples were also required to be positive by a second immunoassay (Roche or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG antibody, we assessed 350 plasma samples from participants classed as 'known negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from individuals confirmed to be negative across all three laboratory assays (Roche, EuroImmun, Abbott). Using these positive n=304 and negative n=350 antibody cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the AbC-19 LFIA (Table 2).

Given a recent report of lower specificity in the AbC-19 LFIA (17) and the possibility of introducing sample bias, we revised our inclusion criteria for the negative cohort. For the pre-pandemic cohort, we included samples from all 223 individuals, regardless of results on other laboratory immunoassays. When this assumed negative pre-pandemic cohort was used for laboratory evaluation for target condition of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 2). We expanded the negative cohort to include all samples that matched our criteria (samples collected during the pandemic to be negative by all three laboratory assays and all pre-pandemic samples regardless of other immunoassay results). The specificity observed on this extended negative cohort of 488 samples was 99.59% (98.53% to 99.95%, Table 2). For sensitivity analysis on a positive cohort (samples positive by EuroImmun and one other test), we were able to analyse all samples previously untested due to limited testing capacity and tested a positive cohort of 330 samples giving a sensitivity of 97.58% (95.28% to 98.95%, Table 2). When we sorted samples analysed in both negative (n=488) and positive cohorts (n=330) by

RT-PCR status and assessed AbC-19 LFIA sensitivity by including only those that were RT-PCR positive (n=227), the test showed a sensitivity of 92.07% (87.76%-95.23%, Table S3, Figure S3b). However, of the n=18 RT-PCR positive individuals negative for IgG antibodies by AbC-19, n=12 showed no detectable antibodies by all three laboratory assays (EuroImmun, Roche or Abbott), suggesting that antibodies are not present in those samples (Figure S3c).

When used as intended by the public, the AbC-19 LFIA provides binary positive/negative results. However, when assessing LFIA in the laboratory, each test line was scored against a scorecard by three independent researchers (0 negative, 1-10 positive; Figure S2). When compared to quantitative outputs from the Abbott, EuroImmun and Roche assays, the AbC-19 LFIA shows good correlation (Abbott r=0.84 [p<0.001]; EuroImmun r=0.86 [p<0.001]; Roche r=0.82 [p<0.001]; Figure 3, Figure S7-Figure S9).

Analytical specificity and sensitivity of AbC-19 LFIA

We observed no cross-reactivity across samples with known H5N1 influenza, Respiratory syncytial virus, Influenza A, Influenza B, Bordetella Pertussis, Haemophilus Influenzae, Seasonal coronavirus NL63 and 229E on the AbC-19 LFIA (n=34 samples, n=8 distinct respiratory viruses; Table S4). Against a panel of external reference SARS-CoV-2 serology samples, the AbC-19 LFIA detected antibodies with scores commensurate to the EuroImmun ELISA scores (Figure S10, Table S5).

Discussion

Serological antibody immunoassays are an important tool in helping combat the SARS-CoV-2 pandemic. The duration of the humoral immune response is of particular importance, to inform an individual's protection following both natural infection and vaccination. Using a large cohort of individuals across a wide age range (18-78 years), we assessed antibody levels across up to three laboratory immunoassays perform a cross-sectional and longitudinal analysis over time. Our results show strong correlation between all three immunoassays, with shortcomings in the Abbott system output 0.25-1.4 range, as described previously, suggesting an overestimated positive cut-off (Figure 1) (15).

Longitudinal studies on SARS-CoV-1 convalescent patients suggests that detectable IgG can still be present as long as 2 years after infection (18). There are conflicting reports of the longevity of the humoral response to SARS-CoV-2 infection which differ in the make-up of the cohort studied, the assays used, and the length of time since symptom onset. The longevity of IgG antibodies to both spike and nucleocapsid protein more than 10 months after RT PCR positive status (and beyond in a small number of samples, Figure 2, Figure S6) is consistent with that observed in other recent studies(19–21). In this study, samples were collected through a convalescent plasma program (Figure S6), with individuals selected for sequential plasma donation based on an initial high EuroImmun assay score. In contrast to the time series analysis of healthcare workers recruited prospectively by Manisty *et al*., we observed no cases where Euroimmun ELISA-measured anti-Spike antibody levels fell below threshold, whilst a large number of Abbott measured anti-Nucleocapsid antibody levels dropped below the positivity threshold (34.7% 26/75). However, this may be an overestimate given the shortcomings of the Abbott assay

described above (Figure 1) (22). In a similar longitudinal study of 51 symptomatic participants, Dan *et al*. estimated that half-life ($t_{1/2}$) for IgG-Spike (103 days) was longer than that for IgG-Nucleocapsid (68 days), although with a considerable overlap of 95% confidence intervals (23).

In our more diverse cross-sectional cohort, we also note a statistically significant decline over time but levels remain detectable at 140 days (Figure 2). We note that IgG levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as Week 8-12 from first symptoms or a viral RNA RT-PCR positive result, though this may be an artefact of lower number of participants at earlier timepoints (Table 1). Robust antibody responses are produced in our cohorts across a wide age range (18-78 years old, Figure 2, Figure S6). We detect a slightly but significantly lower median age of participants testing positive (Figure S4); however, this is likely be due to cohort characteristics and not a true reflection of the population or indication of test performance.

A difficulty faced in validation of antibody diagnostic assays has been access to samples with known SARS-CoV-2 antibody status. As previously described, there is no clear gold standard reference against which to assess SARS-CoV-2 immunoassays. A positive RT-PCR test has been used previously to indicate previous (COVID-19) SARS-CoV-2 infection, though this approach is limited by a high rate of false negatives and positives in RT-PCR testing, failure in some cases to develop IgG antibodies (sero-silence or lack of antibody against the same antigenic component of the virus as the immunoassay uses as a capture antigen) and the lack of RT-PCR testing availability early in the pandemic (3,5,24). SARS-CoV-2 IgG antibodies were undetectable in 14 of 267 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA

positive participants in this study. It is unclear if this is due to insufficient/absent antibody production in these individuals at the time the sample was taken, or due to a false positive PCR result which may occur in the UK at a rate between 0.8- 4.0% (6). Self-assessment of symptoms for COVID-19 (disease) is a poor indicator of previous infection, even amongst healthcare workers (25). Additionally, the kinetics of a SARS-CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus with time, contributing to false negative RT-PCR test results for individuals who may be late to present for virus detection tests (5,26).

To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-CoV-2 antibody in a laboratory evaluation, we developed a reference standard for SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar approach was used in a recent seroprevalence study in Iceland, whereby two positive antibody results were required to determine a participant sample as positive for SARS-CoV-2 antibody (24). Our evaluation of performance metrics for the UK-RTC AbC-19 LFIA to detect antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59% specificity. In an evaluation of the AbC-19 tests, Mulchandani *et al*. observed a specificity of 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report a sensitivity of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a previous RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys antibody test, which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid portion of SARS-CoV-2 (25). In RT-PCR positive individuals from our cohorts, the AbC-19 test showed a similar sensitivity (92.07%, 87.76%- 95.23%, Table S3). However, we demonstrate the drawbacks of this approach given that in 12 of 18 AbC-19 false negatives, none of the four immunoassays used (EuroImmun, Roche, Abbott

or AbC-19) detected antibodies, suggesting either a false RT-PCR result, a failure to produce IgG antibodies or sero-reversion before sample collection in these individuals. Another recent evaluation of the AbC-19 LFIA by Moshe *et al*. determined a sensitivity of (100% (98.1-100%) on laboratory sera, using a composite reference standard of antibody positive by either Spike protein ELISA or hybrid DABA assay and specificity of 99.8% (98.9-100%) against pre-pandemic samples. However, when AbC-19 performance was analysed on matched finger-prick and serum samples against the same antibody standard, a lower sensitivity was observed (finger-prick 69% (53.8-81.3%), serum 92% (80-97.7%)) (27).

In our study, strong correlation was observed in quantitative score between results on all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA (Figure S8, S9). This is to be expected, given both the AbC-19 LFIA and EuroImmun ELISA detect IgG antibodies against spike protein. Importantly, for the assessment of immunity to prior natural infection as well as to immunisation, IgG antibodies against SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA and AbC-19 LFIA are known to correlate with neutralizing antibodies, which may confer future immunity (23,28,29). Previous evaluations of sensitivity and specificity reported by Public Health England (PHE), showed a EuroImmun sensitivity of 72% and specificity of 99%, Abbott with sensitivity of 92.7% and specificity of 100% and Roche with sensitivity of 83.9% and specificity of 100% (30–32). The PHE analyses for each of these tests used previous infection (RT-PCR positive status) as a reference standard, the limitations of which are discussed above.

In the use of characterised 'known positive' and 'known negative' cohorts, one limitation of this study is its potential for spectrum bias, whereby our positive-by-two reference system may artificially raise the threshold for positive sample inclusion, possibly resulting in the overestimation of the sensitivity of any test evaluated (33). However, similar issues have been raised when using previous RT-PCR result or definitive COVID-19 symptoms as inclusion criteria given these will likely skew a cohort towards more severe disease, especially given issues of RT-PCR availability outside of hospital settings during the first wave (5). Importantly, our mixed origin of samples forming the cohort provides a positive cohort for assessing assay sensitivity that includes individuals from the general public, healthcare workers and from convalescent plasma programmes. In the absence of a clear gold standard test, our system relies on no single test (each with their individual shortcomings) and instead takes an average of three. Our analysis of specificity on only pre-pandemic individuals (n=223) shows similar specificity (99.55%) to the larger mixed 'known negative cohort' (n=488, sensitivity 99.59%). We also demonstrate a high level of analytical specificity of the AbC-19 test with no cross-reactivity against a panel of other respiratory viruses, including SARS-CoV-1 NL63 and 229E (Table S4).

Our assessment of the AbC-19 LFIA in a laboratory setting, using characterised cohorts of known SARS-CoV-2 antibody positive and antibody negative plasma, shows good performance metrics for its ability to detect SARS-CoV-2 IgG antibodies following natural infection. We note our use of plasma from venous blood samples, as opposed to a finger prick blood sample as would be used in rapid testing scenarios (27). Additionally, when the AbC-19 LFIA was used on our cohort, a number of the positive results scored low (1/10 using the score card under laboratory conditions,

Figure 3), with a faint test band visible to a trained laboratory scientist but perhaps difficult to identify as positive by individuals performing a single test (Figure S10). This faint line may be reflective of the longer time from infection for the Northern Ireland cohort used. If the AbC-19 LFIA is to be used in clinical settings it is important to determine if all users observe the same results as observed in this laboratory evaluation.

This assessment of the AbC-19 LFIA does not provide data on how this test will perform in a seroprevalence screening scenario, but instead provides metrics for the performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as opposed to previous COVID-19 infection. An important potential use of the AbC-19 LFIA would be in monitoring the immune response to vaccination, with most vaccines utilising SARS-CoV-2 Spike protein antigens (34).

Conclusion

We present a comprehensive analysis of pre-pandemic and two large pandemic cohorts (more than 700 individuals) and in a longitudinal analysis showing that IgG antibodies to SARS-CoV-2 antigens are detectable more than 10 months from positive RT-PCR test. We use antibody positive status as an alternative to RT-PCR positive status as a standard for assessing SARS-CoV-2 antibody assays and show strong performance for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-CoV-2 antibodies. User experience in future studies in the real world is important and may alter the performance characteristics. Also, the effect of operator training will have direct effects upon test performance. We welcome further clinical evaluation of the AbC-19 LFIA in large cohorts of symptomatic and asymptomatic individuals alongside

large studies assessing vaccination outcomes in individuals to fully validate its implementation across all intended use cases.

Declarations

Ethics approval and consent to participate

All study participants provided informed consent. This study was approved by Ulster University Institutional Ethics committee (REC/20/0043), South Birmingham REC (The PANDEMIC Study IRAS Project ID: 286041Ref 20/WM/0184) and adhered to the Declaration of Helsinki and Good Clinical Practice.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Consent for publication

Not applicable.

Dissemination to participants and related patient and public communities.

Links to this work will be included on the study website (https://www.ulster.ac.uk/coronavirus/research/research-output/pandemic-study) and participants will be alerted that the work has been published.

Data sharing

Data are available on reasonable request to the corresponding author.

Competing interests:

At the time of this study TM and JML acted as advisors to CIGA HealthCare, an industrial partner in the UK Rapid Test Consortium. No personal financial reward or renumeration was received for this advisory role. At the time of submission of this manuscript TM and JML no longer held these advisory positions.

All other authors have no potential conflict of interest to report.

Funding:

Costs for assays and laboratory expenses only will be paid by UK-RTC as is normal practice (UU-UK-RTC-2020-001). The authors have not been paid or financially benefitted from this study.

The advisory roles within CIGA Healthcare were unpaid temporary roles. This manuscript and associated data within this paper has only been used to build confidence into the overall device design and performance assessment of the UK RTC AbC-19 devices and such work was never commissioned for any government contractual consideration.

Authors' contributions:

TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR, SM and KYN analysed data, KB performed all statistical analyses/interpretations and produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and provided Blood Transfusion cohort samples. TM, RP and AN coordinated participant recruitment, consent and sampling. WB and JML developed online consent forms, questionnaires and databases. LR, JM, AK, AA, GEW, DH, SS, CCS performed sample collection and processing. LR and TM wrote the manuscript, with significant contributions from JM, AN and KB. All authors reviewed and approved the final manuscript.

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Table 1: Antibody level ratios for assays over time

Antibody level ratios for assays over time show varying peak levels depending on test. Calculated by first establishing the median per time period, then calculating log2 ratio for each period versus each respective assay positivity cut-off.

	Ratio median antibody level: assay positivity cut-off									
	Week									
	Pre-2020	1-2	3-4	5-8	9-12	13-16	18-20	21-24	25-28	29+
EuroImmun	-2.65	1.33	0.2	0.95	1.32	0.47	0.04	-2.01	-2.26	-2.01
Roche	-3.64	3.16	3.05	5.20	5.45	4.14	4.42	-3.54	-3.69	-3.61
Abbott	-5.54	1.64	-0.51	1.12	0.86	0.08	-0.59	-5.13	-5.13	-6.13
Sample number (n=)	223	20	10	52	90	202	53	11	12	11

Table 2: UK-RTC AbC-19 LFIA performance metrics against known antibody positive and known antibody negative cohorts.

Total Negative	True Negative	False Positive	Total Positive	True Positive	False Negative	Sensitivity % (95 CI)	Specificity % (95 CI)
Pre-pandemic (n=223)							
223	222	1	n/a	n/a	n/a	n/a	99.55% (97.53% to 99.99%)
Initially reported cohorts (n=654)							
350	350	0	304	297	7	97.70% (95.31%-99.07%)	100.00% (98.95%-100.00%)
Extended cohorts (n=818)							
488	486	2	330	322	8	97.58% (95.28%-98.95%)	99.59% (98.53%-99.95%)

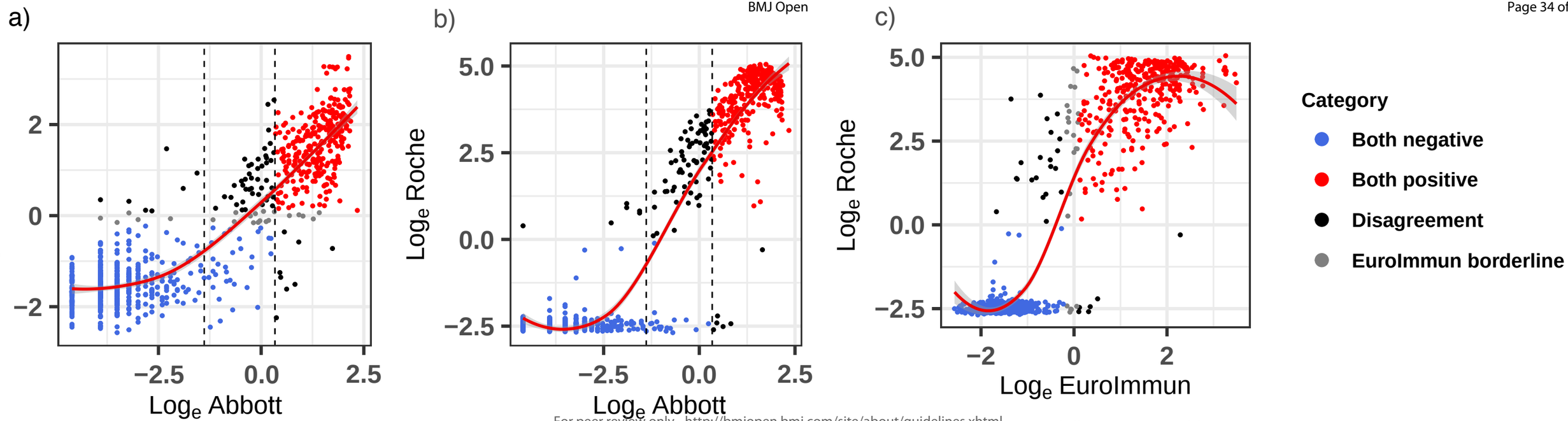
Figure Legends

Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott and c) Roche immunoassays. Pearson χ^2 test was used to assess correlations. The results for each test were log transformed to ensure results follow a normal distribution. Negative agreement shown as blue dots, red dots show positive agreement for the two immunoassays, whilst black dots show disagreement and grey dots as the EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4. n=880. The graphs show positive correlations between all immunoassays evaluated, with the fewest disagreement of results between the Log of Roche and the Log of EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.

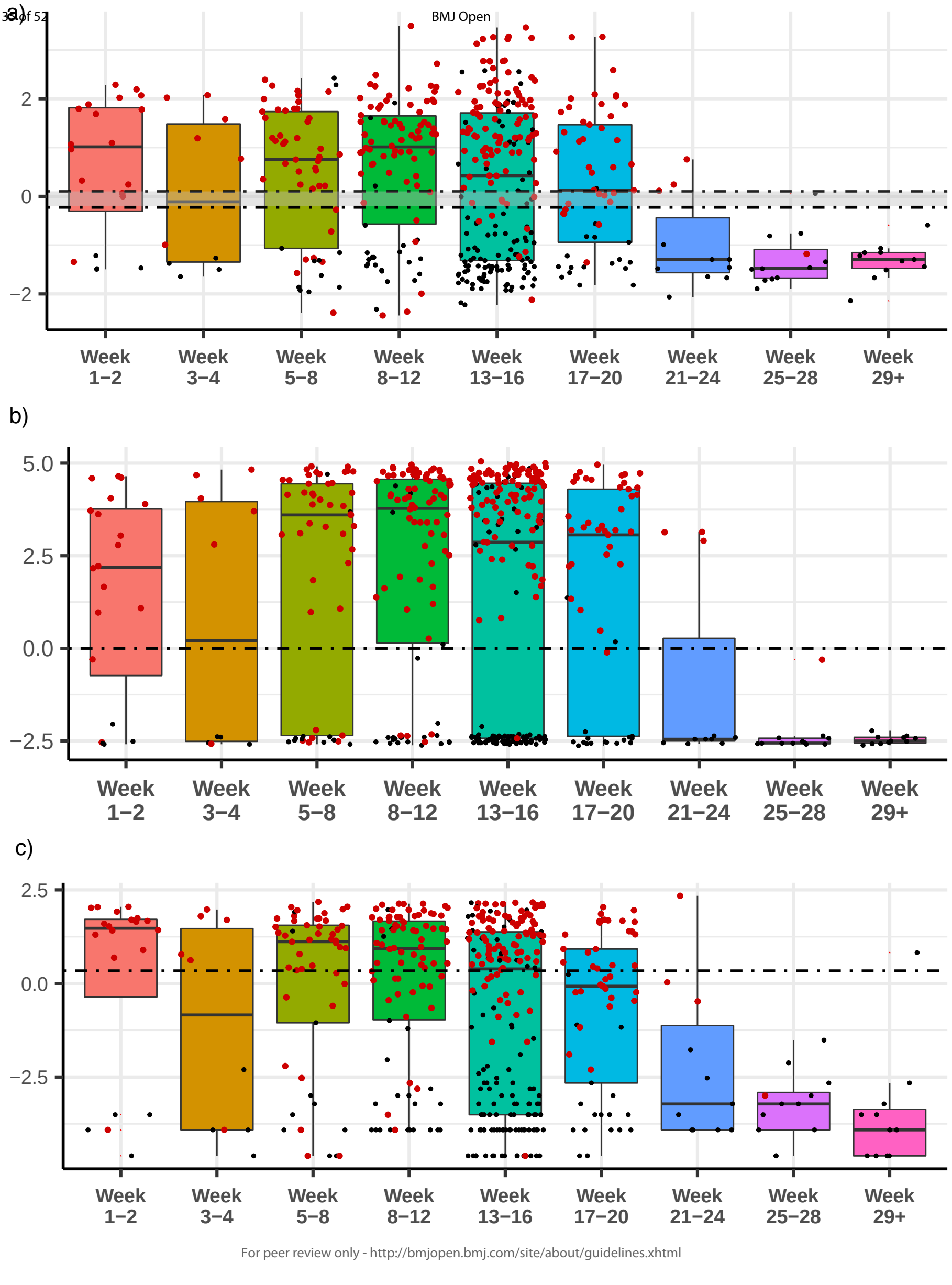
Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c) Abbott, relative to weeks since first reported symptoms or positive PCR result (where data available, n=685). RT-PCR positive individuals are denoted by red dots, while individuals with time since symptom data are denoted in black. Dashed lines delineate $\log_e$ equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result between the two lines). Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).

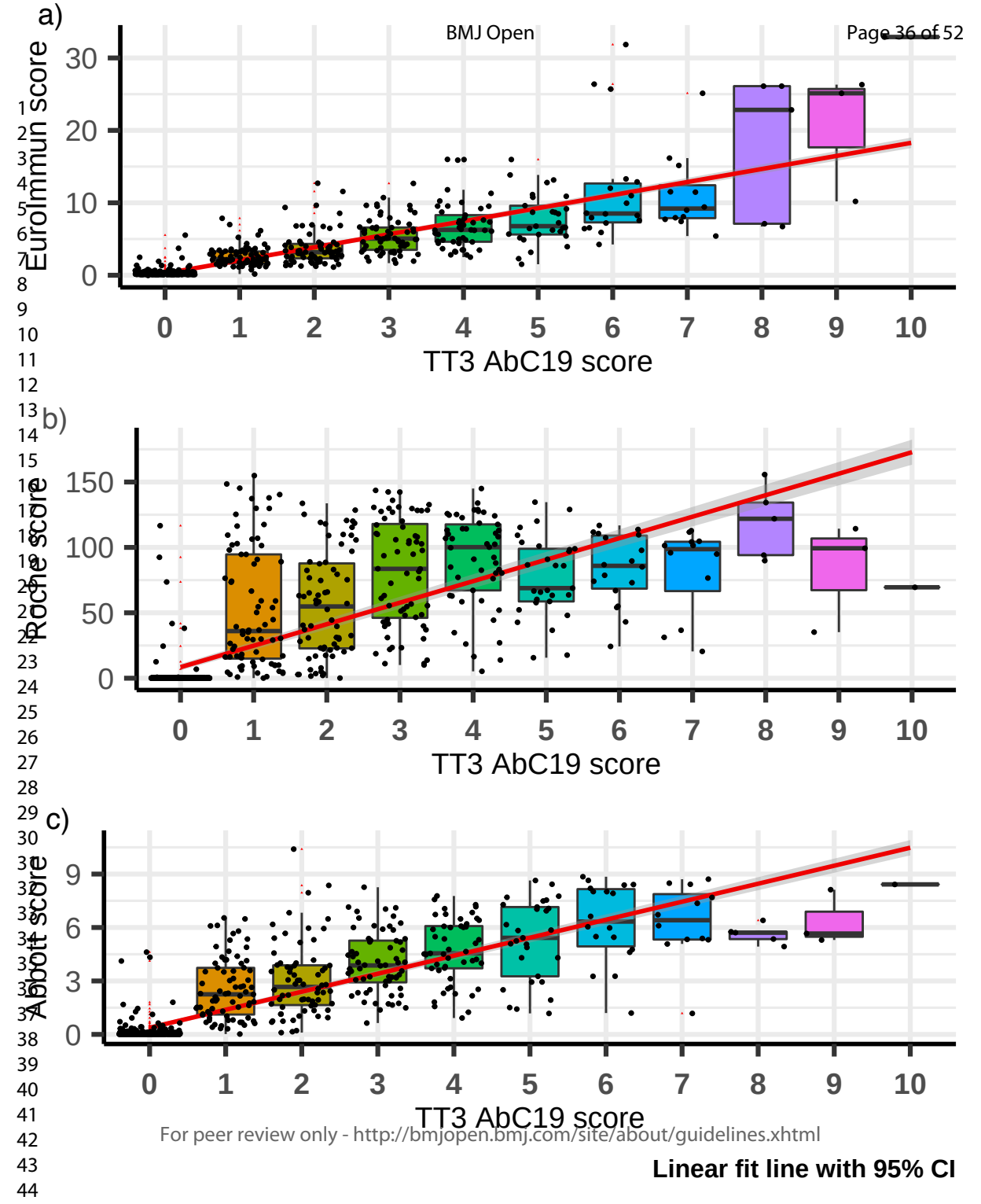
Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing AbC-19 TT3 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line

of best fit with 95% confidence interval shaded in grey. Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on 1.5* IQR (interquartile range).



Fit lines, LOESS with 95% CI
Vertical lines mark Abbott test range 0.25−1.4


a)
Log$_e$ EuroImmun
2
0
−2
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+
b)
Log$_e$ Roche
5.0
2.5
0.0
−2.5
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+
c)
Log$_e$ Abbott
2.5
0.0
−2.5
Week 1–2
Week 3–4
Week 5–8
Week 8–12
Week 13–16
Week 17–20
Week 21–24
Week 25–28
Week 29+



Linear fit line with 95% CI

Supplementary Materials

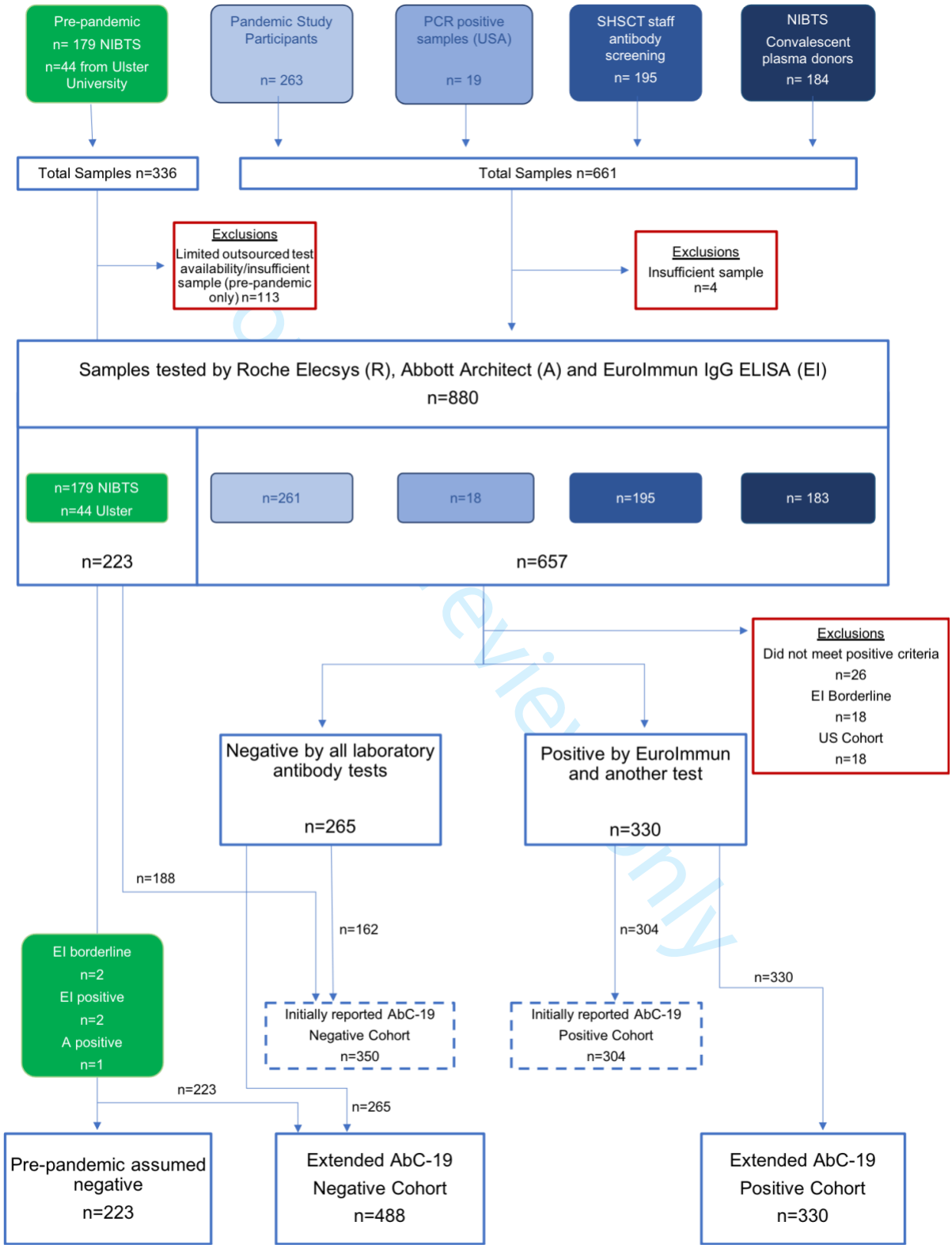
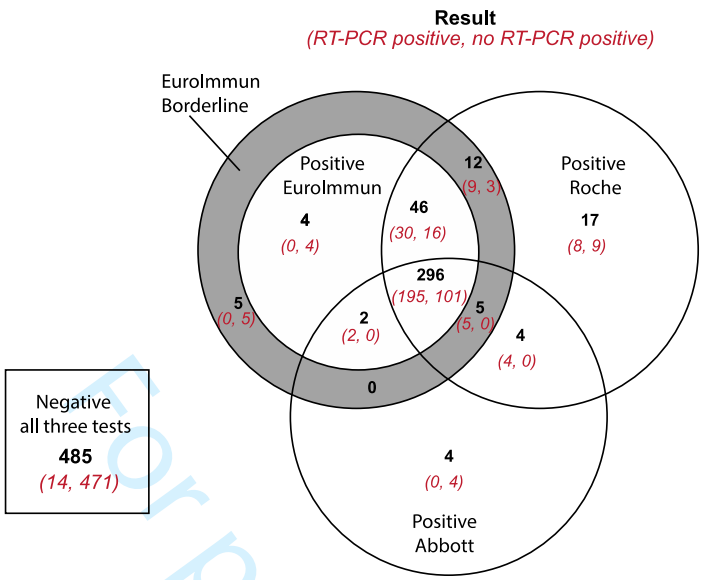


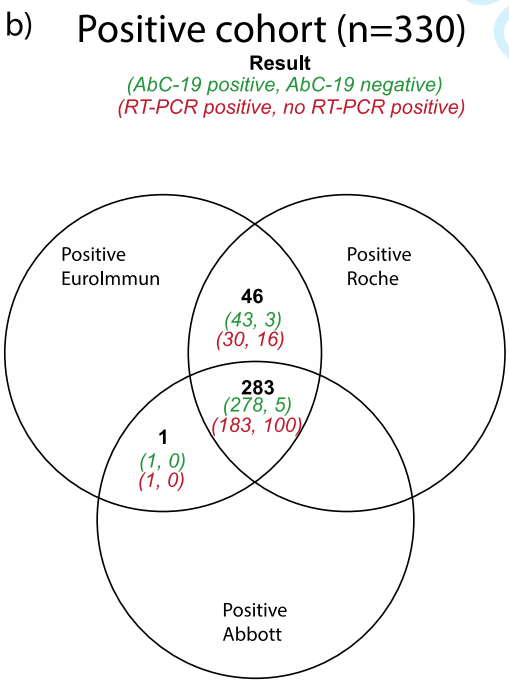
Figure S1: Flow of participant plasma samples through cross-sectional study. All available samples from participants within each cohort, and the included and excluded samples at all stages. Freeze thaw cycles were closely monitored for all sample aliquots. Pre-pandemic samples taken forward for Roche, Abbott and EuroImmun testing were selected based on aliquot volume and availability.

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

a)



b)



c)

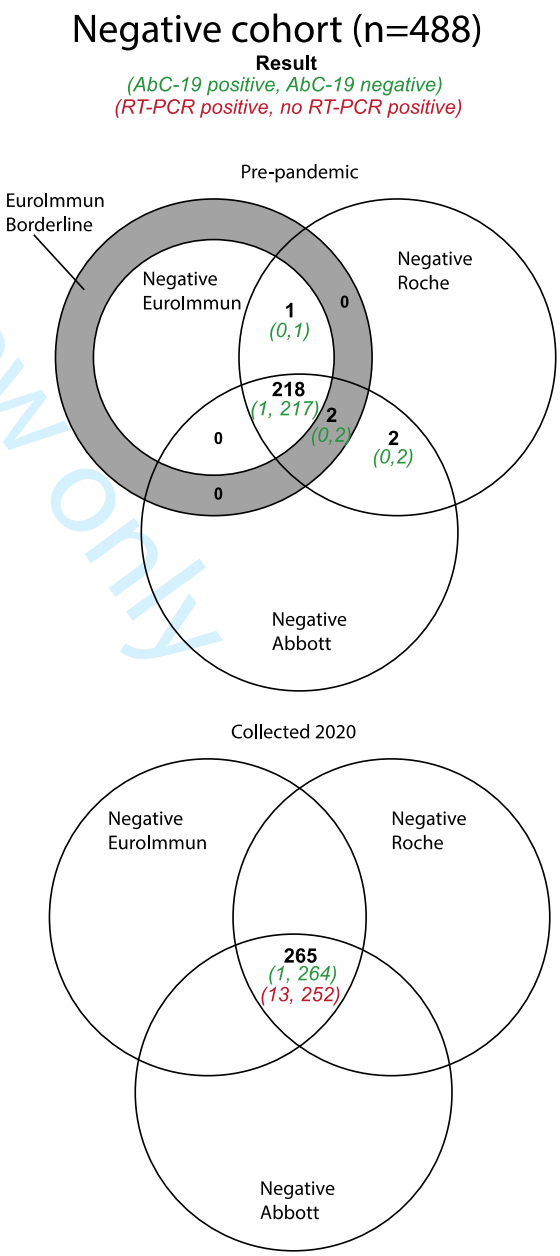


Figure S3: Venn diagrams demonstrating result overlap between laboratory assays in a) the initial immunoassay cohort (n=880), b) the positive and c) negative cohorts assessed with AbC-19 TT3. Result in each circle overlap in bold, (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below.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

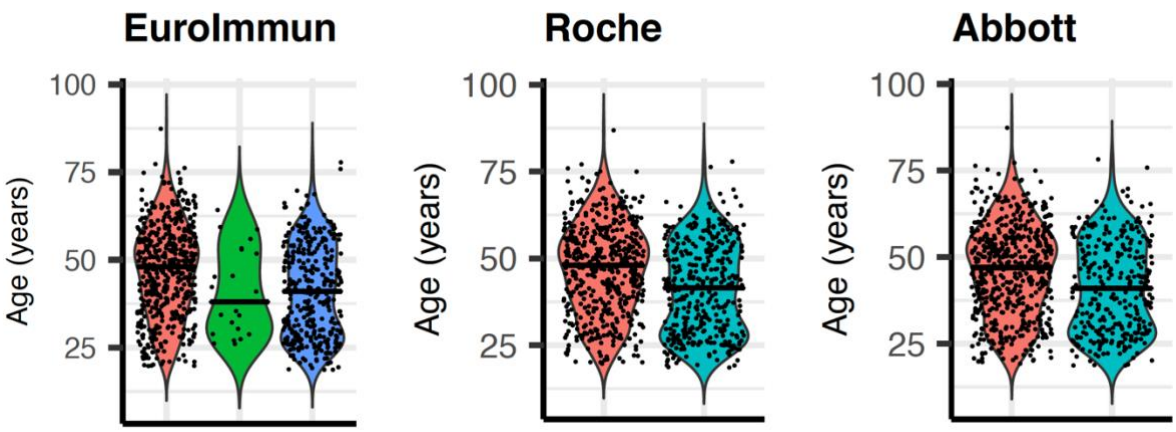


Figure S4: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.

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

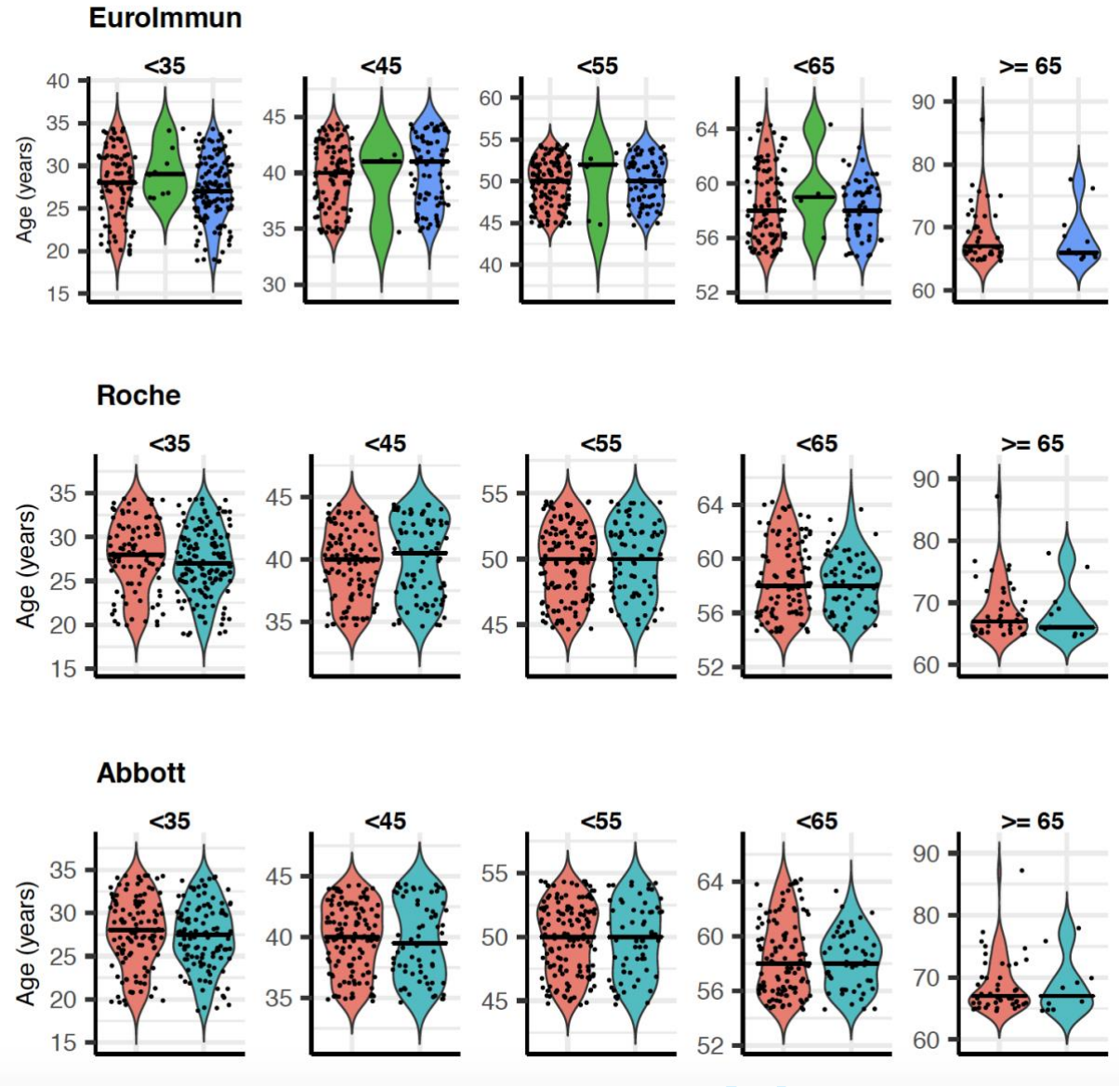


Figure S5: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.

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

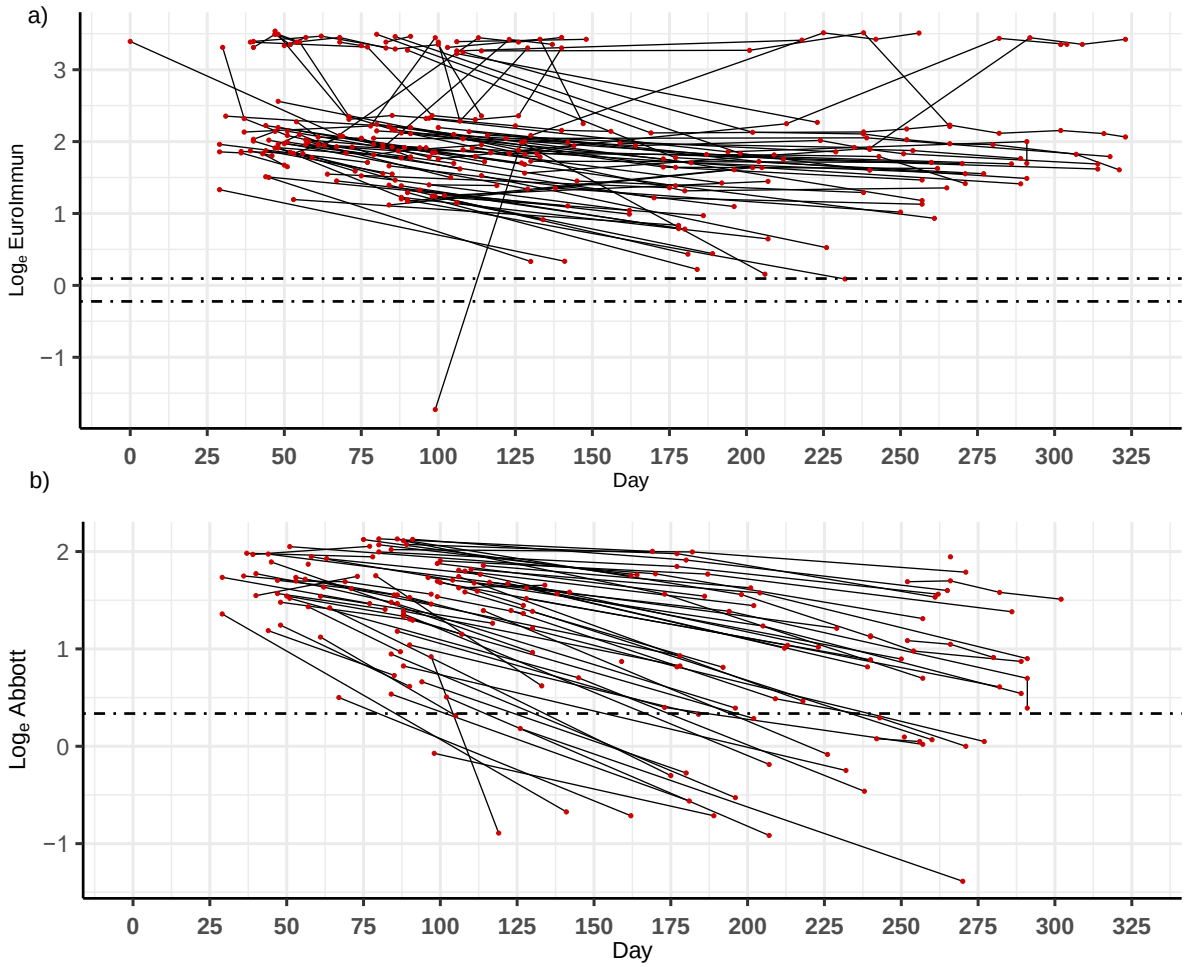


Figure S6: Longitudinal analysis of convalescent plasma donor sequential samples (2-9 samples per individual) by a) EuroImmun ELISA or b) Abbott immunoassay. a) n=101 individuals, grey shading indicates borderline region, upper dotted line indicates positivity threshold (1.1), lower dotted line indicates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

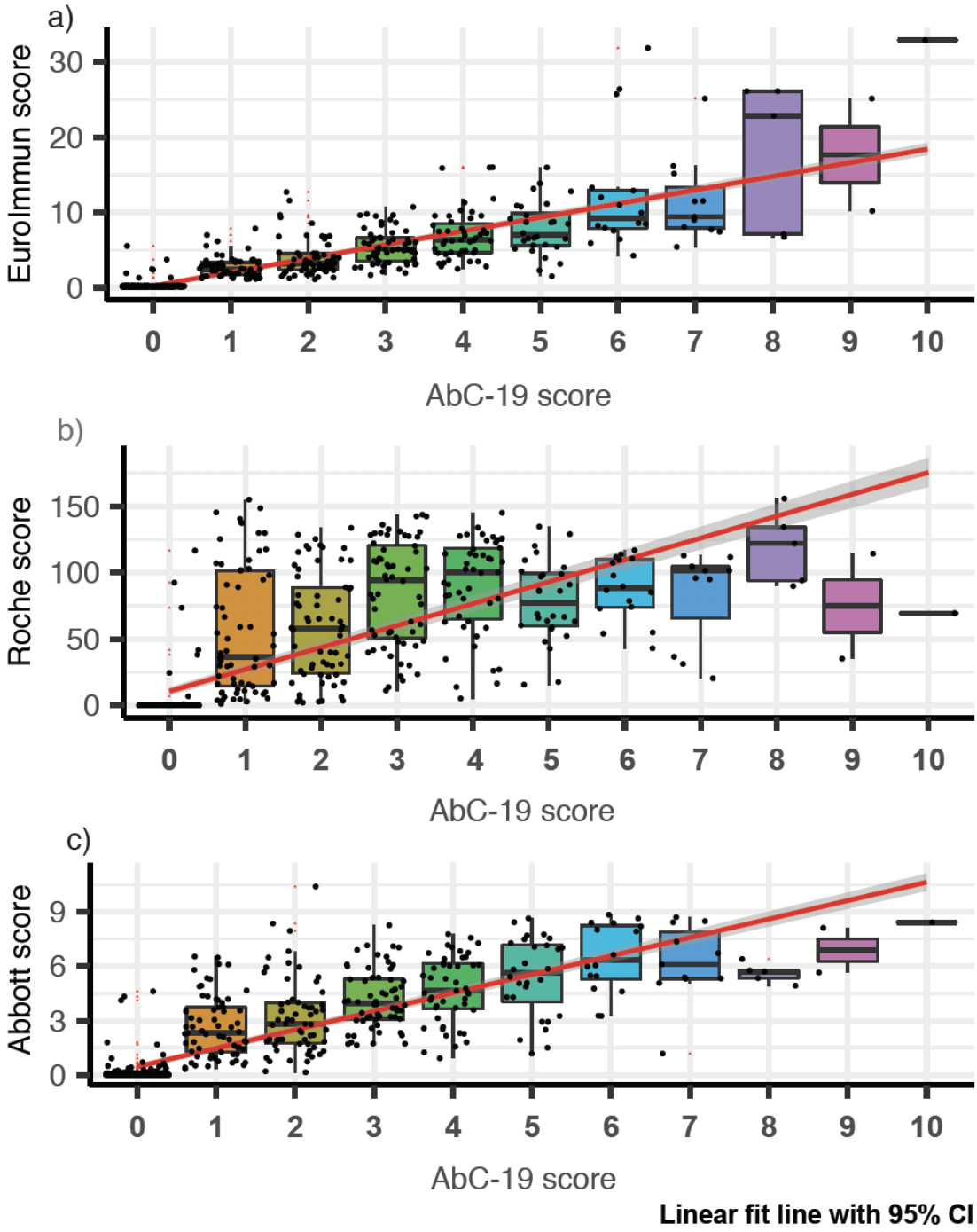


Figure S7: AbC-19 initially reported cohort n=654 correlation to a) EuroImmun b) Roche and c) Abbott scores. Box plots overlaid on scatter plot, comparing TT3 AbC-19 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line of best fit with 95% confidence interval shaded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

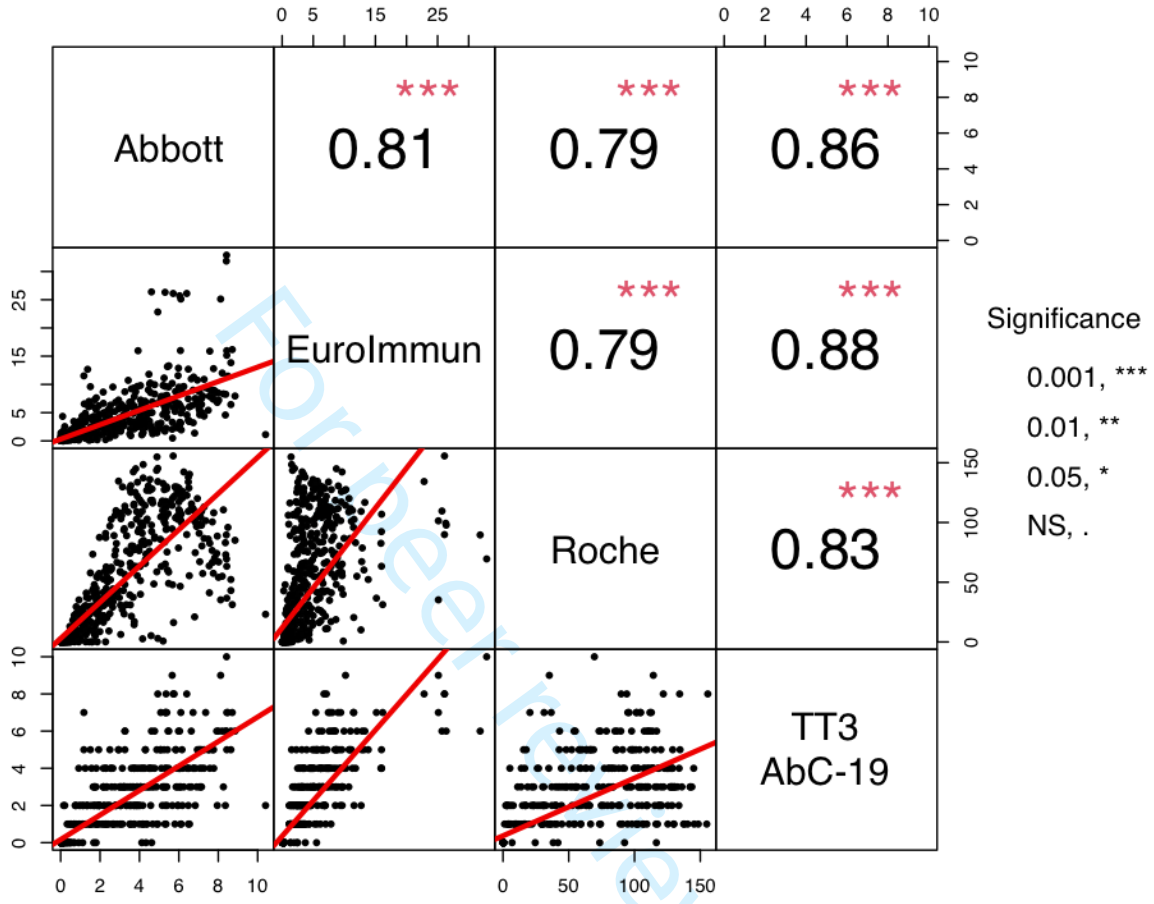


Figure S8: **Correlation matrix between Abbott, EuroImmun, Roche and initially reported AbC-19 cohort (n=654) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly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

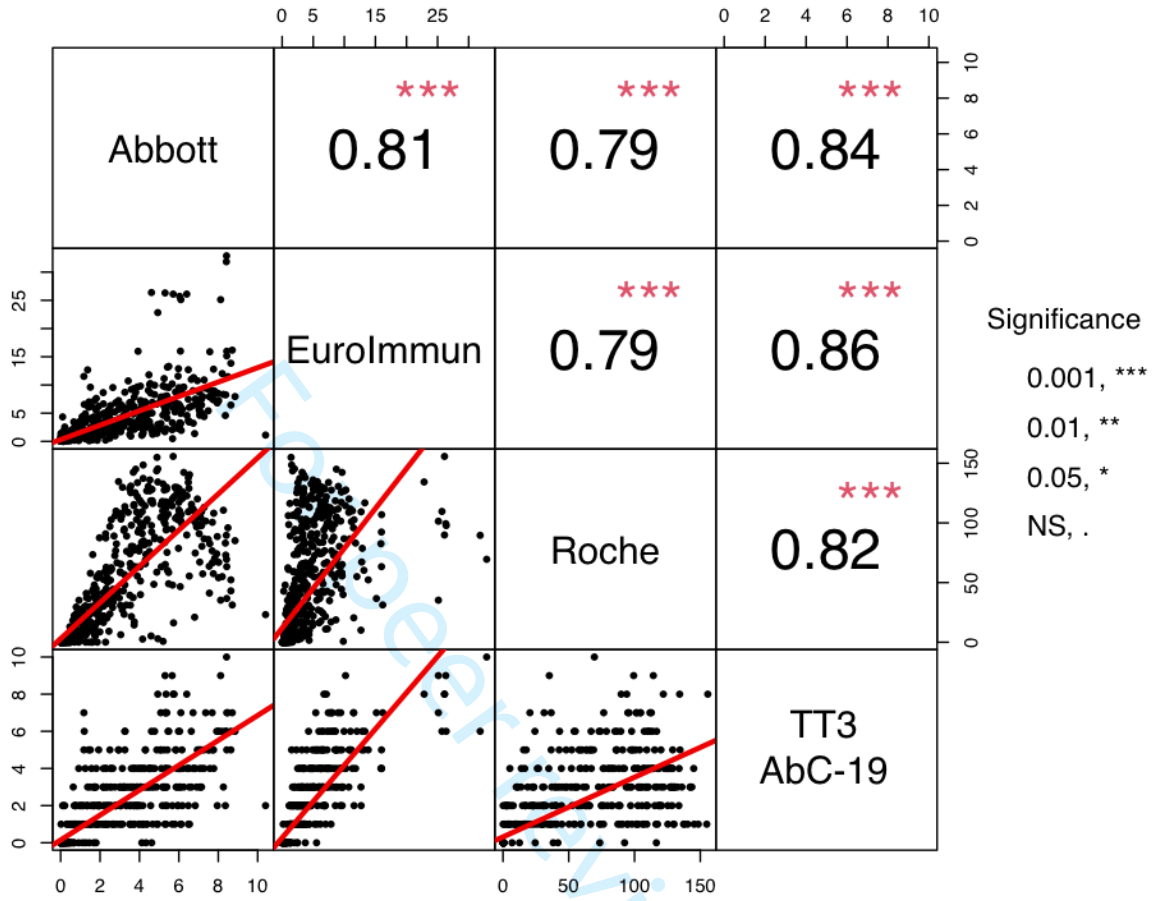


Figure S9: **Correlation matrix between Abbott, EuroImmun, Roche and extended AbC-19 cohort (n=818) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at $p<0.05$. All immunoassays were significantly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

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

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

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

Immunoassay	Principle	Antigen Target	Assay Time (min)	Antibody Detected	Measurement	Result	Calibration	Evaluation of results	Results
EuroImmun ELISA	Enzyme-linked immunosorbent assay (enzyme-HRP)	S1 domain of the spike protein	120	IgG	Photometric measurement of the color intensity using wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm	OD (Optical density)	One Positive calibrator	OD of clinical sample/OD of calibrator	< 0.8 Negative, ≥ 0.8 to <1.1 Borderline, ≥ 1.1 Positive
Roche Elecsys immunoassay	Electro-chemiluminescence	Nucleocapsid	18	IgG, IgA and IgM	Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier	RLU (Relative Light Intensity)	One Positive calibrator and one Negative calibrator	The analyzer automatically calculates the cut-off based on the measurement of ACOV2 Cal1 (negative) and ACOV2 Cal2 (positive). The result of a sample is given either as reactive or non-reactive as well as in the form of a cut-off index (COI; signal sample/cut-off).	< 1.0 Negative, ≥ 1.0 Positive
Abbott Architect SARS-CoV-2	Chemiluminescent microparticle immunoassay	Nucleocapsid	30	IgG	The resulting chemiluminescent reaction is measured as a relative light unit (RLU).	RLU (Relative Light Intensity)	One Positive calibrator	Results are reported by dividing the sample result by the calibrator result (mean of 3 calibrators). The default result unit for the SARS-CoV-2 IgG assay is Index (S/C).	< 1.4 Negative, ≥ 1.4 Positive
IT3 AbC-19	Rapid Point of Care Lateral Flow Immunoassay	Full length Spike protein	20	IgG	The colour intensity of the test line is analysed using the reference score card.	Binary	The presence of a control line indicates the test is valid.	A result is positive if there is both a test line and a control line, whilst a result is negative if only the control line is present.	Using the reference score card; Positive scores ≥1 Negative scores=0

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

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

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

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

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

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

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

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

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

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

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

Supplementary Methods

Laboratory-based immunoassays

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

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

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

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

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

Analytical specificity and sensitivity assessment

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

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5-6
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
Participants	**6**	Eligibility criteria	7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6/7
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6/7
	9	Whether participants formed a consecutive, random or convenience series	6
Test methods	**10a**	Index test, in sufficient detail to allow replication	8
	10b	Reference standard, in sufficient detail to allow replication	8
	11	Rationale for choosing the reference standard (if alternatives exist)	5-6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	8, supp table 1
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Supp methods, supp table 1
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Supp methods
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8/9
	15	How indeterminate index test or reference standard results were handled	11, Supp Fig1
	16	How missing data on the index test and reference standard were handled	Supp Fig 1
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	12-14
	18	Intended sample size and how it was determined	8-9
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Supp Fig 1
	20	Baseline demographic and clinical characteristics of participants	10
	21a	Distribution of severity of disease in those with the target condition	10
	21b	Distribution of alternative diagnoses in those without the target condition	10
	22	Time interval and any clinical interventions between index test and reference standard	6-7
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Fig 3, Fig S3, S5-S7
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	12-13, Table 1
	25	Any adverse events from performing the index test or the reference standard	n/a
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	4, 18-20
	27	Implications for practice, including the intended use and clinical role of the index test	19-20
OTHER INFORMATION			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents

	30	Sources of funding and other support; role of funders	22