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# BMJ Open

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

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Keywords:	COVID-19, Molecular diagnostics < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES

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

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## 36 **Abstract**

### 37 *Objective*

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

### 42 *Design*

43 Nationwide serological study.

### 44 *Setting*

45 Northern Ireland, UK, May - August 2020.

### 46 *Participants*

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

### 51 *Main Outcome Measures*

52 SARS-CoV-2 antibody levels in plasma samples using Roche Elecsys Anti-SARS-  
53 CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2  
54 ELISA immunoassays over time. UK-RTC AbC-19 LFIA sensitivity and specificity,  
55 estimated using a reference standard system to establish a characterised panel of  
56 330 positive and 488 negative SARS-CoV-2 IgG samples.

### 57 *Results*

58 We detected persistence of SARS-CoV-2 IgG up to 140 days (20 weeks) post  
59 infection, across all three laboratory-controlled immunoassays. On the known positive  
60 cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58%  
61 (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-  
62 99.95%).

### 63 *Conclusions*

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

69

**70 Strengths and Limitations**

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

77

78 Strength - This study demonstrates AbC-19 lateral flow point of care detection of IgG  
79 antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the antibodies made  
80 in response to the vaccines used globally, in a large cohort of subjects, 330 positive  
81 samples, up to 140 days post infection, across a broad age range (18-90 years).  
82 Robust antibody responses were observed in all age groups tested, including over-  
83 65s, who are most at risk of severe COVID-19 symptoms, with the eldest in our  
84 population having priority for mass vaccination.

85

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

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

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3 94 using a reference standard alongside one other assay which would allow for the  
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5 95 possibility of spectrum bias and may well not reflect the true performance metrics of  
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7 96 any of the assays evaluated when translated to real life settings, using finger prick  
8  
9 97 blood samples, in which pre-test probability would impact greatly on positive and  
10  
11 98 negative predictive values.  
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## 100 **Keywords**

101 SARS-CoV-2, COVID-19, immunoassay, LFIA, pandemic, antibody assay  
102

## 103 **Introduction**

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

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

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



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3 119 and assessing responses to vaccination programmes. The timing for when antibody  
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5 120 against the novel SARS-CoV-2 virus can be measured is at this time not fully  
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8 121 characterised.

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10 122 Commercial serology immunoassays are mostly laboratory-based and measure IgG  
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13 123 antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIAs),  
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15 124 require a finger prick blood sample and can be used at point-of-care (POC) or in the  
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17 125 home; particularly important in the context of lockdown enforcement during the  
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20 126 pandemic. Currently, a limited number of laboratory-based chemiluminescence  
21  
22 127 immunoassays are approved for use in the UK including the Roche Elecsys Anti-  
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24 128 SARS-CoV-2 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region  
25  
26 129 (Roche Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay  
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28  
29 130 against the same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

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31 131 The complexities of the humoral immune response to SARS-CoV-2 is a much-  
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33  
34 132 debated topic. In a US study, approximately one in 16 individuals lacked detectable  
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36 133 IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR  
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38 134 confirmed infection (7). Patients who remain asymptomatic may mount a humoral  
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41 135 immune response which is short-lived, with detectable levels of antibody falling  
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43 136 rapidly (8). This, alongside potentially low sensitivity and lack of RT-PCR test  
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45 137 availability across the UK has hindered development of well characterised gold  
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48 138 standard serology test for IgG antibodies to SARS-CoV-2.

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50 139 Herein, we describe the use of Roche and Abbott commercial immunoassays, as well  
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52 140 as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike  
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54 141 antigenic protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-  
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56 142 pandemic and pandemic COVID-19 blood samples (n=880) from within Northern  
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59 143 Ireland and report on longevity of IgG antibodies detected. Presently, there is no gold  
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3 144 standard assay for comparison, therefore we aimed to establish a reference based on  
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5 145 a positive COVID-19 antibody status. We present results of a laboratory evaluation of  
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7 146 the UK-RTC AbC-19 with a target condition of antibodies against a cohort of 330  
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9 147 known IgG antibody positive samples according to this 'positive by two' system and  
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11 148 488 negative samples (223 pre-pandemic assumed negative and 265 known negative)  
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13 149 for IgG to SARS-CoV-2.  
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## 151 **Methods**

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### 153 **Participant samples**

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

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3 169 collection of 263 10ml EDTA plasma samples from 263 separate study participants.  
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5 170 Additional anonymised plasma samples were obtained from Southern Health and  
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7 171 Social Care Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood  
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9 172 Transfusion Service (NIBTS, n=184) through convalescent plasma programs.  
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14 174 Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster  
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16 175 University ethics committee approved studies with ongoing consent and from NIBTS  
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18 176 (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-  
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20 177 thaw cycles for all analyses reported within this manuscript.  
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### 25 179 **Clinical information**

26  
27 180 Basic demographic information and data with regard to probable or definite prior  
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29 181 infection with SARS-CoV-2 virus was obtained from PANDEMIC study participants  
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31 182 through the secure online questionnaire requiring responses about positive RT-PCR  
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33 183 result and/or time from symptom onset. Anonymised participant samples from USA,  
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35 184 SHSCT and NIBTS were provided with age, gender and time since PCR-positive,  
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37 185 where a previous test had been carried out.  
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### 43 187 **Laboratory-based immunoassays**

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45 188 Details of laboratory immunoassays are summarised in supplementary methods and  
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47 189 Table S1.  
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### 52 191 **UK-RTC AbC-19 LFIA**

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54 192 UK-RTC AbC-19 testing was conducted at Ulster University according to  
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56 193 manufacturer's instructions (details in Table S1). Assays were performed as cohorts,  
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3 194 with samples in batches of 10, with one researcher adding 2.5µL of plasma to the  
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5 195 assay and a second adding 100µL of buffer immediately following sample addition.  
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8 196 After 20 minutes, the strength of each resulting test line was scored from 0-10  
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10 197 according to a visual score card (scored by 3 researchers; Figure S2). A score  $\geq 1$  was  
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12 198 positive. Details of samples used for analysis for detection of antibodies are available  
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15 199 in Supplementary methods.  
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200

### 201 **Statistical analysis**

202 As per Daniel (9) a minimum sample size based on prevalence can be calculated  
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24 203 using the following formula:  $n = \frac{Z^2 P(1-P)}{d^2}$ , where n = sample size, Z = Z statistic for a  
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27 204 chosen level of confidence, P = estimated prevalence, and d = precision. Assuming  
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29 205 a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the  
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31 206 required sample size at 99% confidence (Z = 2.58) to be 240 individuals. If the true  
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33 207 prevalence is lower, 5%, the estimated required sample size given a precision of  
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35 208 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200  
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37 209 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody  
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40 210 immunoassays(10).

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43 211 Statistical analysis was conducted in R v 4.0.2(11). To assess discordance between  
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45 212 test results, data was first filtered to include individuals with an Abbott test result in the  
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48 213 range  $\geq 0.25$  &  $\leq 1.4$ , with a 2 x 2 contingency table produced that comprised all  
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50 214 possible combinations of [concordant|discordant] test results [within|outside of] this  
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52 215 range. A p-value was derived via a Pearson  $\chi^2$  test after 2000 p-value simulations via  
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54 216 the stats package.

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57 217 AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc  
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59 218 Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To

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3 219 compare test result (Positive|Negative) to age, a binary logistic regression model was  
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5 220 produced with test result as outcome – a p-value was then derived via  $\chi^2$  ANOVA. To  
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7 221 compare time against test result (encoded continuously), a linear regression was  
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9 222 performed. We calculated median per time-period and then converted these to log  
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11 223 [base 2] ratios against the positivity cut-off for each assay. All plots were generated  
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13 224 via ggplot2 or custom functions using base R(12).  
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## 226 **Results**

227 We analysed samples from a mixed cohort of individuals from the general public  
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24 228 (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations  
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26 229 and research studies (n=223) and through a convalescent plasma program (n=183).  
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28 230 Antibody levels in plasma from these 880 individuals were assessed using the three  
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30 231 SARS-CoV-2 immunoassays; EuroImmuno IgG, Roche Elecsys IgG/IgM/IgA and  
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32 232 Abbott Architect IgG (Table S1). This included a cohort of 223 pre-pandemic plasma  
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34 233 samples collected and stored during 2017 to end of May 2019 to determine assay  
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36 234 specificity. Of the 657 participants whose samples were collected during the  
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38 235 pandemic, 265 (40.33%) previously tested RT-PCR positive with a range of 7-173  
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40 236 days since diagnosis. A total of 225 participants gave time since self-reported COVID-  
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42 237 19 symptoms, with a range of 5-233 days from symptom onset, whilst 198 had no  
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44 238 symptom or PCR data available.  
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### 240 *Laboratory based antibody immunoassays*

241 A positive result for antibody on one or more of the three laboratory immunoassays  
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26 242 was recorded for 385/657 (58.6%) participants who provided a sample during the  
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3 243 pandemic. By EuroImmune ELISA, 346 were positive, 20 borderline and 291 were  
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5 244 negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott  
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7 245 determined 310 positive and 347 negative (Table S2). The median age across all age  
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9 246 groups combined was lower for participants testing positive across each of the  
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11 247 immunoassays (median [sd] for positive versus negative, respectively: EuroImmune, 41  
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13 248 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41 [13.18] vs 47 [13.09]).  
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15 249 (Figure S3,  $p < 0.0001$ ). When segregated by age group, however, differences were  
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17 250 less apparent in certain groups (Figure S4). Excluding the pre-pandemic cohort, this  
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19 251 gap reduced but remained statistically significant EuroImmune, 41 [13.18] vs 45 [12.49];  
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21 252 Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41 [13.26] vs 44 [12.63]) ( $p < 0.01$ ) (median  
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23 253 [sd] for positive versus negative). Of note, out of 265 individuals with a previous  
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25 254 positive RT-PCR result for SARS-CoV-2 viral RNA, 14 (5.2%) did not show detectable  
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27 255 antibodies by any of the three immunoassays, with no association found with age,  
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29 256 gender or time between test and blood draw (data not shown).

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36 257 The three commercial laboratory immunoassays provide a ratio value that increases  
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38 258 with IgG antibody titre. When correlation between these values is assessed, good  
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40 259 overall agreement is observed between the three immunoassays (Figure 1, Figure  
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42 260 S5). As highlighted by Rosadas *et al.*, we also see significant disagreement in the  
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44 261 Abbott 0.25-1.4 range when compared to EuroImmune and Roche (Figure 1a,b; chi-  
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46 262 square p-values: EuroImmune vs Abbott,  $p < 0.001$ ; Roche vs Abbott,  $p < 0.001$ )(13).

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#### 51 52 264 *Duration of humoral response to SARS-CoV-2*

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54 265 We found IgG antibodies could still be detected in individuals (excluding pre-  
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56 266 pandemic) across all three immunoassays used up to week 20 (day 140) (Figure 2).  
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59 267 We note a statistically significant decrease in signal with respect to time across each

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3 268 assay (p-value [slope]): EuroImmuno, p=0.036 [-0.785]; Roche, p=0.002 [-0.125];  
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5 269 Abbott, p<0.0001 [-3.585]. These remained statistically significant after adjustment  
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8 270 for age. Antibody levels (expressed as a ratio of median result per timepoint divided  
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10 271 by positivity cut off; Table 2) peaked at Week 1-2 for EuroImmuno (1.33) and Abbott  
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12 272 (1.64), though reached highest levels at Week 8-12 when measured by Roche  
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14 273 (5.45). By week 21-24, median score for all tests had dropped below the positivity  
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17 274 cut off, though a small number of RT-PCR positive samples remained above the  
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19 275 positive cut off at these later timepoints (Figure 2).  
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### 23 24 277 *UK-RTC AbC-19*

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26 278 Using the commercial immunoassays described we established a well characterised  
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28 279 serology sample set of 'known positive' and 'known negative' for IgG antibodies to  
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30 280 SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.  
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33 281 AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore  
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35 282 required all samples to be positive by the EuroImmuno SARS-CoV-2 IgG ELISA, which  
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37 283 likewise detects antibodies against the S1 domain (14). To develop this characterised  
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39 284 cohort, samples were also required to be positive by a second immunoassay (Roche  
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41 285 or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG  
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43 286 antibody, we assessed 350 plasma samples from participants classed as 'known  
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45 287 negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from  
46  
47 288 individuals confirmed to be negative across all three laboratory assays (Roche,  
48  
49 289 EuroImmuno, Abbott). Using these positive n=304 and negative n=350 antibody  
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51 290 cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of  
52  
53 291 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the  
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55 292 AbC-19 LFIA (Table 1).  
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3 293 Given a recent report of lower specificity in the AbC-19 LFIA (15) and the possibility  
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5 294 of introducing sample bias, we revised our inclusion criteria for the negative cohort.  
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7 295 For the pre-pandemic cohort, we included samples from all 223 individuals,  
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9 296 regardless of results on other laboratory immunoassays. When this assumed  
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11 297 negative pre-pandemic cohort was used for laboratory evaluation for target condition  
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13 298 of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 1). We  
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15 299 obtained more AbC-19 devices and expanded the negative cohort to include all  
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17 300 samples that matched our criteria (samples collected during the pandemic to be  
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19 301 negative by all three laboratory assays and all pre-pandemic samples regardless of  
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21 302 other immunoassay results). The specificity observed on this extended negative  
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23 303 cohort of 488 samples was 99.59% (98.53% to 99.95%, Table 1). For sensitivity  
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25 304 analysis on a positive cohort (samples positive by EuroImmun and one other test),  
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27 305 we were able to analyse all samples previously untested due to limited testing  
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29 306 capacity and tested a positive cohort of 330 samples giving a sensitivity of 97.58%  
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31 307 (95.28% to 98.95%, Table 1).  
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40 309 When used for its intended use case, the AbC-19 LFIA provides binary  
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42 310 positive/negative results. However, when assessing LFIA in the laboratory, each test  
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44 311 line was scored against a scorecard by three independent researchers (0 negative, 1-  
45  
46 312 10 positive; Figure S2). Compared to quantitative outputs from the Abbott, EuroImmun  
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48 313 and Roche assays, the AbC-19 LFIA shows good correlation (Abbott  $r=0.84$  [ $p<0.001$ ];  
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50 314 EuroImmun  $r=0.86$  [ $p<0.001$ ]; Roche  $r=0.82$  [ $p<0.001$ ]; Figure 3, Figure S5-Figure S7).  
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56 316 *Analytical specificity and sensitivity of AbC-19 LFIA*  
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3 317 We observed no cross-reactivity across samples with known H5N1 influenza,  
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5 318 Respiratory syncytial virus, Influenza A, Influenza B, Bordetella Pertussis,  
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7 319 Haemophilus Influenzae, Seasonal coronavirus NL63 and 229E on the AbC-19 LFIA  
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9 320 (n=34 samples, n=8 distinct respiratory viruses; Table S3). Against a panel of external  
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11 321 reference SARS-CoV-2 serology samples, the AbC-19 LFIA detected antibodies with  
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13 322 scores commensurate to the EuroImmune ELISA scores (Figure S8, Table S4).  
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## 16 324 **Discussion**

17 325 Serological antibody immunoassays are an important tool in helping combat the  
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19 326 SARS-CoV-2 pandemic. One difficulty faced in validation of antibody diagnostic  
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21 327 assays has been access to samples with known SARS-CoV-2 antibody status. As  
22  
23 328 previously described, there is no clear gold standard for reference against which to  
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25 329 assess SARS-CoV-2 immunoassays. A positive RT-PCR test has been used  
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27 330 previously to indicate previous COVID-19 infection, though this approach is limited by  
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29 331 a high rate of false negatives, failure in some cases to develop IgG antibodies (sero-  
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31 332 silence or lack of antibody against the same antigenic component of the virus as the  
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33 333 immunoassay uses as a capture antigen) and the lack of RT-PCR testing availability  
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35 334 early in the pandemic (3,5,16). We failed to detect SARS-CoV-2 IgG antibody in 14 of  
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37 335 265 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA positive participants in this  
38  
39 336 study. It is unclear if this is due to insufficient/absent antibody production in these  
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41 337 individuals, or due to a false positive PCR result which may occur in the UK at a rate  
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43 338 between 0.8- 4.0% (17). Self-assessment of symptoms for COVID-19 disease is a  
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45 339 poor indicator of previous infection, even amongst healthcare workers (18).  
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47 340 Asymptomatic individuals may be unaware of infection and others may harbour pre-  
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49 341 existing immunity or elucidate a T cell response. Additionally, the kinetics of a SARS-  
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3 342 CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus  
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5 343 with time, contributing to false negative RT-PCR test results for individuals who may  
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7 344 be late to present for virus detection tests (5,19).  
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12 346 Our results show strong correlation between all three immunoassays, with  
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14 347 shortcomings in the Abbott system output 0.25-1.4 range, as described previously,  
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16 348 suggesting an overestimated positive cut-off (Figure 1) (13). Our detection of  
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18 349 antibodies 140 days after RT PCR positive status (20 weeks, and beyond in a small  
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20 350 number of samples) indicates persistence IgG antibodies to both the spike protein  
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22 351 and nucleocapsid protein, despite typical patterns of antibody decay after acute viral  
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24 352 antigenic exposure being as rapid (20). Others have reported SARS-CoV-2  
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26 353 antibodies decline at 90 days (19), we also noted a statistically significant decline  
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28 354 over time but levels remain detectable at 140 days (Figure 2). We note that IgG  
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30 355 levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as Week 8-  
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32 356 12 from first symptoms or a viral RNA RT-PCR positive result, though this may be an  
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34 357 artefact of lower number of participants at earlier timepoints (Table 2). Longitudinal  
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36 358 studies on SARS-CoV-1 convalescent patients suggests that detectable IgG can still  
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38 359 be present as long as 2 years after infection (21). Further studies are needed on  
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40 360 large cohorts with sequential antibody immunoassays performed on symptomatic  
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42 361 and non-symptomatic individuals as well as those with mild or severe COVID-19 to  
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44 362 fully elucidate the humoral immune response to SARS-CoV-2. This is vital to inform  
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46 363 vaccine durability, so-called 'immune passports' and in the definition of a protective  
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48 364 threshold for anti-SARS-CoV-2 antibodies.  
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3 366 To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-  
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5 367 CoV-2 antibody in a laboratory evaluation, we developed a reference standard for  
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7 368 SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar  
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10 369 approach was used in a recent seroprevalence study in Iceland, whereby two positive  
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12 370 antibody results were required to determine a participant sample as positive for SARS-  
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14 371 CoV-2 antibody (16).

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19 373 Our evaluation of performance metrics for the UK-RTC AbC-19 LFIA to detect  
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21 374 antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59% specificity. In a  
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23 375 recent evaluation of the AbC-19 tests, Mulchandani et al. observed a specificity of  
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25 376 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report a sensitivity  
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27 377 of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a previous  
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29 378 RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys antibody test,  
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31 379 which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid portion of  
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33 380 SARS-CoV-2 (18).

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40 382 In our study, good correlation was observed in quantitative score between results on  
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42 383 all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA  
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44 384 (Figure S6, S7). This is to be expected, given both the AbC-19 LFIA and EuroImmun  
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46 385 ELISA detect IgG antibodies against spike protein. For the assessment of immunity to  
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48 386 prior natural infection as well as to immunisation, it is important to note IgG antibodies  
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50 387 against SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA  
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52 388 and AbC-19 LFIA are known to correlate with neutralizing antibodies, which may  
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54 389 confer future immunity (22,23).

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3 391 Previous evaluations of the sensitivity and specificity reported by Public Health  
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5 392 England (PHE), showed a EuroImmuno sensitivity of 72% and specificity of 99%, Abbott  
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7 393 with sensitivity of 92.7% and specificity of 100% and Roche with sensitivity of 83.9%  
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10 394 and specificity of 100% (24–26). The PHE analyses for each of these tests used  
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12 395 previous infection (RT-PCR positive status) as a reference standard, the limitations of  
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14 396 which are discussed above.

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19 398 In the use of characterised ‘known positive’ and ‘known negative’ cohorts, one  
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21 399 limitation of this study is its potential for spectrum bias, whereby our positive-by-two  
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23 400 reference system may artificially raise the threshold for positive sample inclusion,  
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25 401 possibly resulting in the overestimation of the sensitivity of any test evaluated (27).  
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27 402 However, similar issues have been raised when using previous RT-PCR result or  
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29 403 definitive COVID-19 symptoms as inclusion criteria given these will likely skew a  
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31 404 cohort towards more severe disease (5). Importantly, our mixed origin of samples  
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33 405 forming the cohort provides a positive cohort for assessing assay sensitivity that  
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35 406 includes individuals from the general public, healthcare workers and from  
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37 407 convalescent plasma programmes. Our analysis of specificity on only pre-pandemic  
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39 408 individuals (n=223) shows similar specificity (99.55%) to the larger mixed ‘known  
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41 409 negative cohort’ (n=488, sensitivity 99.59%). In the absence of a clear gold standard  
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43 410 test, our system relies on no single test (each with their individual shortcomings) and  
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45 411 instead takes an average of three.

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51 413 Our assessment of the UK RTC AbC-19 LFIA using our characterised cohorts of  
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53 414 known SARS-CoV-2 antibody positive and antibody negative plasma, in a laboratory  
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55 415 setting shows good performance metrics for its ability to detect SARS-CoV-2 IgG  
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3 416 antibody. We note it uses plasma from venous blood samples, as opposed to the use  
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5 417 of a finger prick blood sample. Additionally, when this UK RTC AbC-19 LFIA was used  
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7 418 on our cohort, a number of the positive results scored low, (1/10 using the score card  
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9 419 under laboratory conditions, Figure 3) with a faint test band visible to a trained  
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11 420 laboratory scientist but perhaps difficult to identify as positive by individuals performing  
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13 421 a single test (Figure S6). This faint line may be reflective of the longer time from  
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15 422 infection for the Northern Ireland cohort used. If this AbC-19 LFIA is to be used in  
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17 423 clinical settings it is important to determine if all users observe the same results as  
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19 424 observed in this laboratory evaluation.  
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26 426 This assessment of the AbC-19 LFIA does not provide data on how this test will  
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28 427 perform in a seroprevalence screening scenario, but instead provides metrics for the  
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30 428 performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as  
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32 429 opposed to previous COVID-19 infection. An important potential use of the AbC-19  
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34 430 LFIA would be in monitoring the immune response to vaccination, with most vaccines  
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36 431 utilising SARS-CoV-2 Spike protein antigens (28). It is not yet known if presence of  
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38 432 SARS-CoV-2 antibodies indicates immunity from infection.  
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## 44 434 **Conclusion**

46 435 We present a comprehensive analysis of 880 pre-pandemic and pandemic individuals  
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48 436 and show IgG antibodies are detectable up to 140 days from symptoms or positive  
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50 437 RT-PCR test, showing persistence of immunity at later time points than previously  
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52 438 published. We use antibody positive as an alternative to RT-PCR positive status as a  
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54 439 standard for assessing SARS-CoV-2 antibody assays and show strong performance  
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56 440 for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-CoV-2  
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3 441 antibodies. It is fully understood that user experience in future studies in the real world  
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5 442 is important and may alter the performance characteristics. Also, the effect of operator  
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7 443 training will have direct effects upon test performance. We welcome further clinical  
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9 444 evaluation of the AbC-19 LFIA in large cohorts of symptomatic and asymptomatic  
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11 445 individuals alongside large studies assessing COVID-19 outcomes in individuals with  
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13 446 longitudinal studies to fully validate its implementation across all intended use cases.  
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## 448 **Declarations**

### 449 **Ethics approval and consent to participate**

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

### 454 **Patient and Public Involvement**

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

### 457 **Consent for publication**

458 Not applicable.

### 459 **Dissemination to participants and related patient and public communities.**

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

### 463 **Data sharing**

464 Data are available on reasonable request to the corresponding author.

### 465 **Competing interests:**

1  
2  
3 466 At the time of this study TM and JML acted as advisors to CIGA HealthCare, an  
4  
5 467 industrial partner in the UK Rapid Test Consortium. No personal financial reward or  
6  
7 468 remuneration was received for this advisory role. At the time of submission of this  
8  
9 469 manuscript TM and JML no longer held these advisory positions.

10  
11  
12 470 All other authors have no potential conflict of interest to report.

13  
14  
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17 472 Costs for assays and laboratory expenses only will be paid by UK-RTC as is normal  
18  
19 473 practice (UU-UK-RTC-2020-001). The authors have not been paid or financially  
20  
21 474 benefitted from this study.

22  
23  
24 475 The advisory roles within CIGA Healthcare were unpaid temporary roles. This  
25  
26 476 manuscript and associated data within this paper has only been used to build  
27  
28 477 confidence into the overall device design and performance assessment of the UK RTC  
29  
30 478 AbC-19 devices and such work was never commissioned for any government  
31  
32 479 contractual consideration.

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34  
35 480 **Authors' contributions:**

36  
37 481 TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR,  
38  
39 482 SM and KYN analysed data, KB performed all statistical analyses/interpretations and  
40  
41 483 produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided  
42  
43 484 SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and  
44  
45 485 provided Blood Transfusion cohort samples. TM, RP and AN coordinated participant  
46  
47 486 recruitment, consent and sampling. WB and JML developed online consent forms,  
48  
49 487 questionnaires and databases. LR, JM, AK, AA, GW, DH, SS, CCS performed  
50  
51 488 sample collection and processing. LR and TM wrote the manuscript, with significant  
52  
53 489 contributions from JM and KB. All authors reviewed and approved the final  
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55 490 manuscript.  
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20  
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12 595 [f](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/890566/Evaluation_of_Abbott_SARS_CoV_2_IgG_PHE.pdf)  
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613 **Table 1: UK-RTC AbC-19 LFIA performance metrics against known antibody**  
 614 **positive and known antibody negative cohorts.**

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

615

616 **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+
<b>EuroImmun</b>	-2.65	1.33	0.2	0.87	1.32	0.47	0.04	-2.01	-2.26	-2.01
<b>Roche</b>	-3.64	3.16	3.05	5.21	5.45	4.14	4.42	-3.54	-3.69	-3.61
<b>Abbott</b>	-5.54	1.64	-0.51	0.99	0.86	0.08	-0.59	-5.13	-5.13	-6.13
<b>Sample number (n=)</b>	223	20	10	50	90	202	53	11	12	11

617

618 Antibody level ratios for assays over time show varying peaks levels depending on  
 619 test. Calculated by first establishing the median per time period, then calculating log<sub>2</sub>  
 620 ratio for each period versus each respective assay positivity cut-off.

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3 622 **Figure Legends**  
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8 624 **Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott**  
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10 625 **and c) Roche immunoassays.** Pearson  $\chi^2$  test was used to assess correlations. The  
11  
12 626 results for each test were log transformed to ensure results follow a normal distribution.  
13  
14 627 Negative agreement shown as blue dots, red dots show positive agreement for the  
15  
16 628 two immunoassays, whilst black dots show disagreement and grey dots as the  
17  
18 629 EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4.  
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21 630 n=880. The graphs show positive correlations between all immunoassays evaluated,  
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23 631 with the fewest disagreement of results between the Log of Roche and the Log of  
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25 632 EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.  
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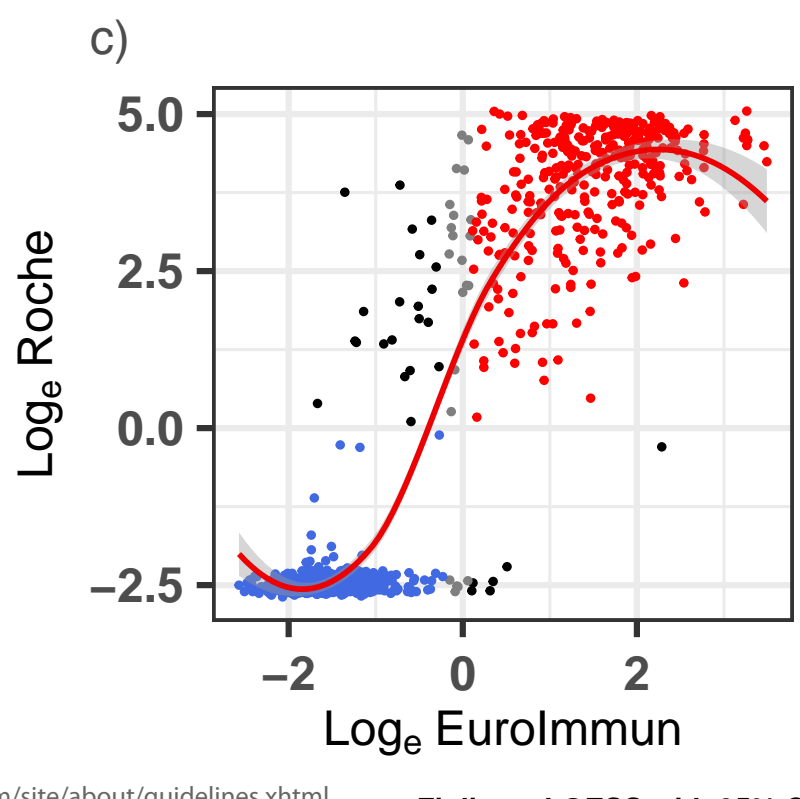
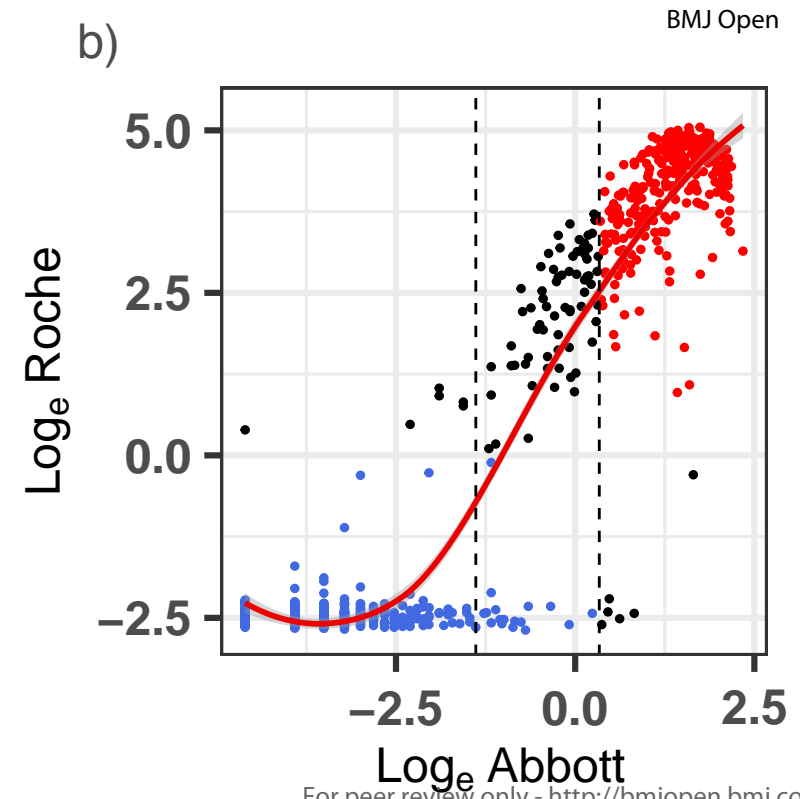
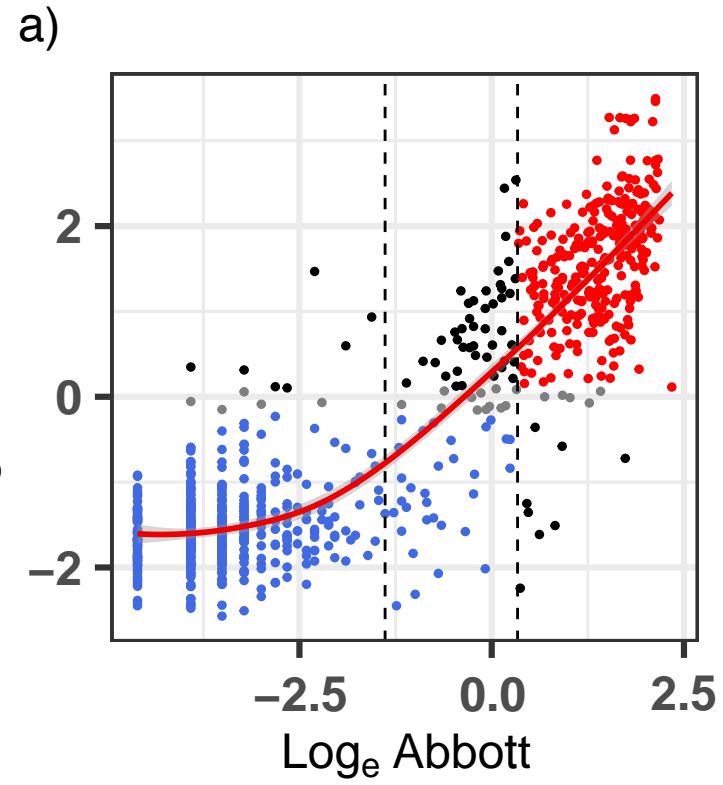
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30 634 **Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c)**  
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32 635 **Abbott, relative to weeks since first reported symptoms or positive PCR result**  
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34 636 **(where data available, n=682).** RT-PCR positive individuals are denoted by red dots,  
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36 637 while individuals with time since symptom data are denoted in black. Dashed lines  
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38 638 delineate  $\log_e$  equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott  
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40 639 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result  
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42 640 between the two lines). Black bars indicate median, within IQR (interquartile range)  
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44 641 boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on  
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46 642  $1.5 \times$  IQR (interquartile range).  
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53 644 **Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche**  
54  
55 645 **and c) Abbott scores.** Box plots overlaid on scatter plot, comparing AbC-19 test  
56  
57 646 scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line  
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3 647 of best fit with 95% confidence interval shaded in grey. Black bars indicate median,  
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5 648 within IQR (interquartile range) boxes for EuroImmuno/Roche/Abbott value. Red  
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8 649 triangles indicate outliers, based on  $1.5 \times$  IQR (interquartile range).  
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- Category**
- Both negative
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  - Disagreement
  - EuroImmun borderline

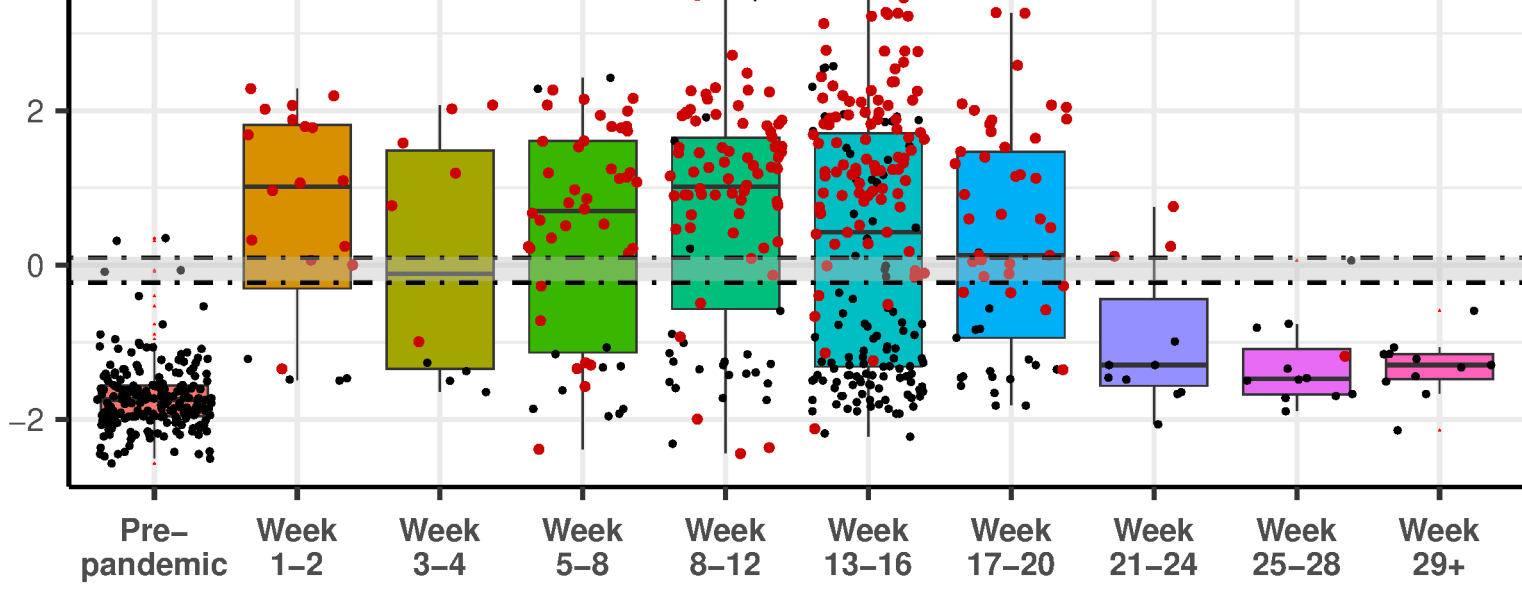
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Fit lines, LOESS with 95% CI  
Vertical lines mark Abbott test range 0.25–1.4



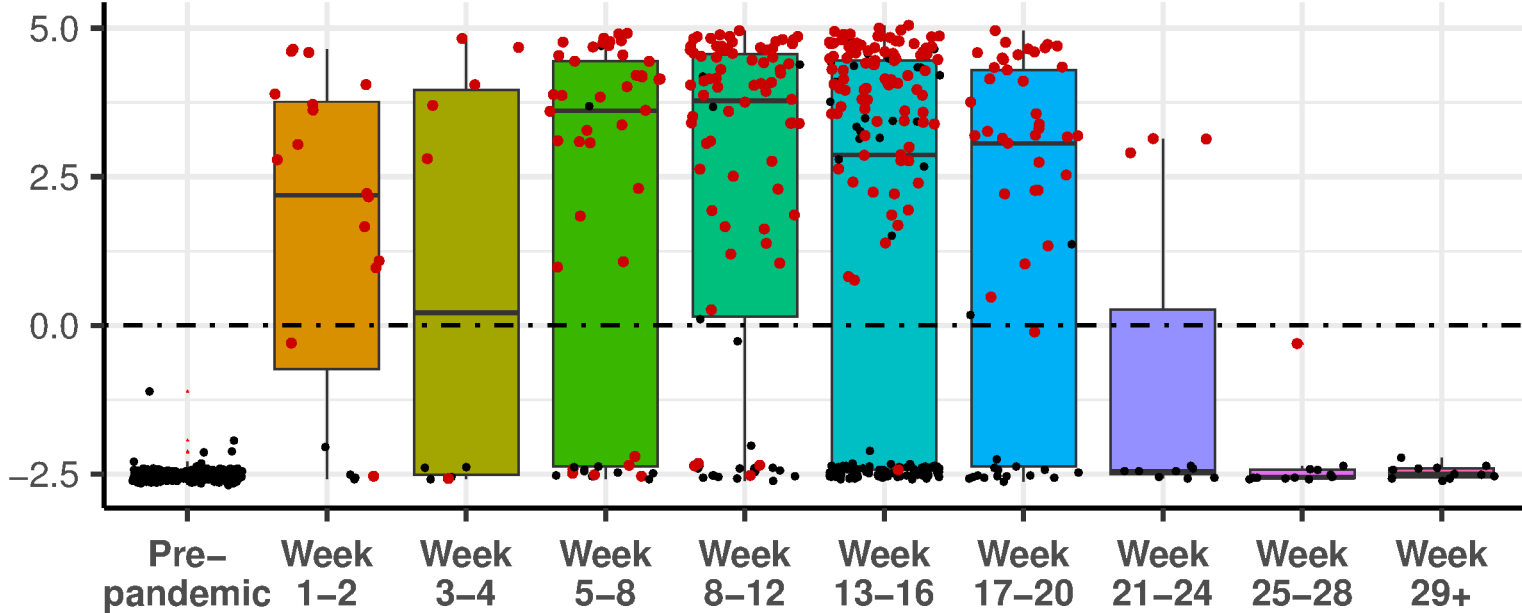
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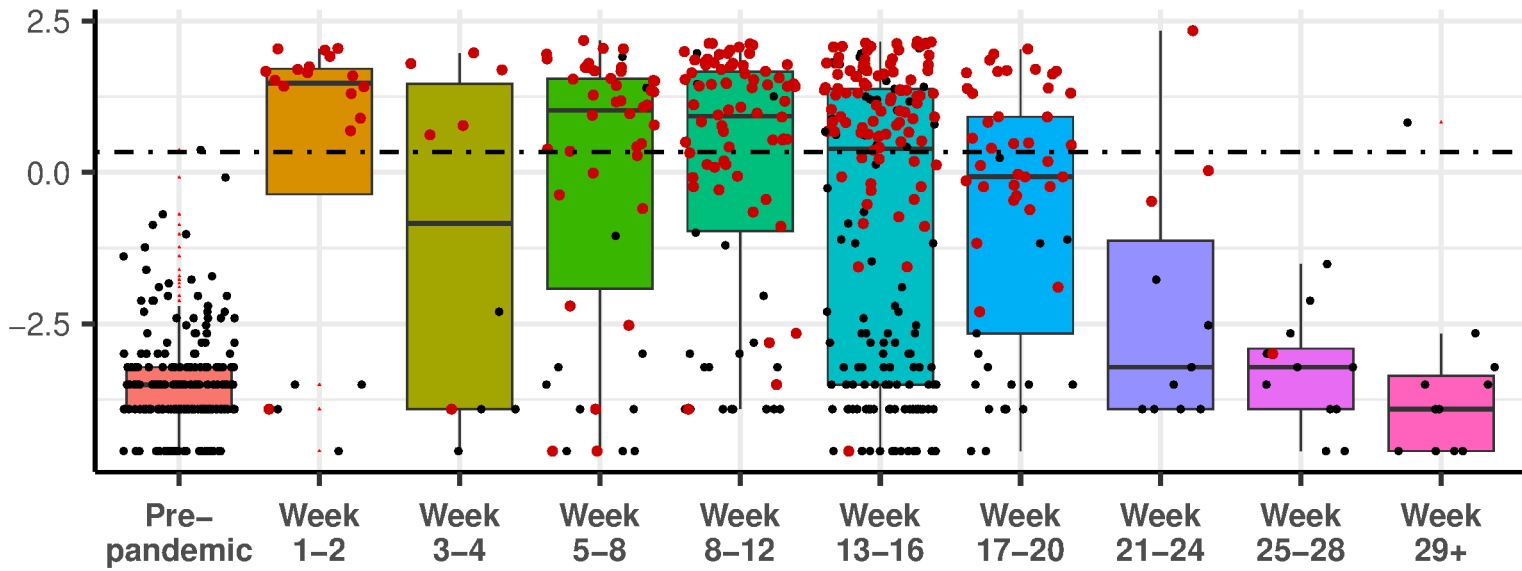
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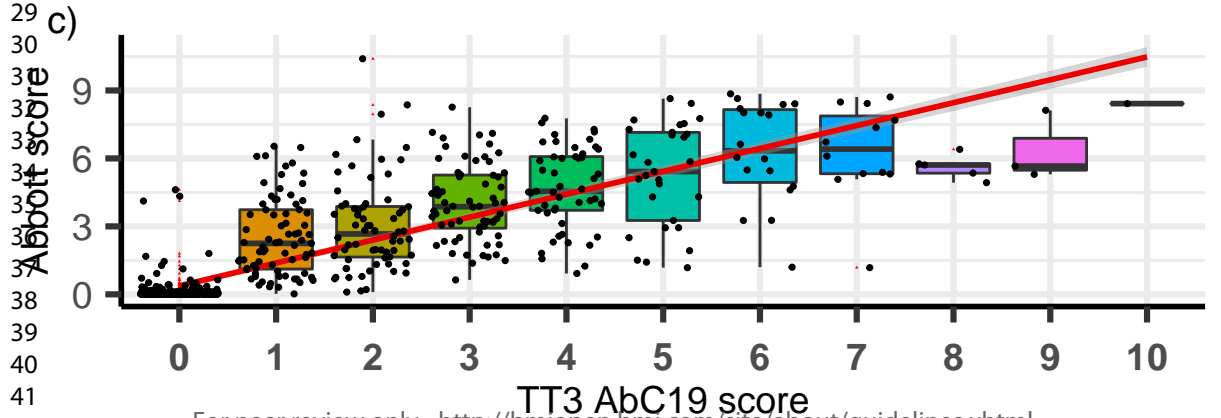
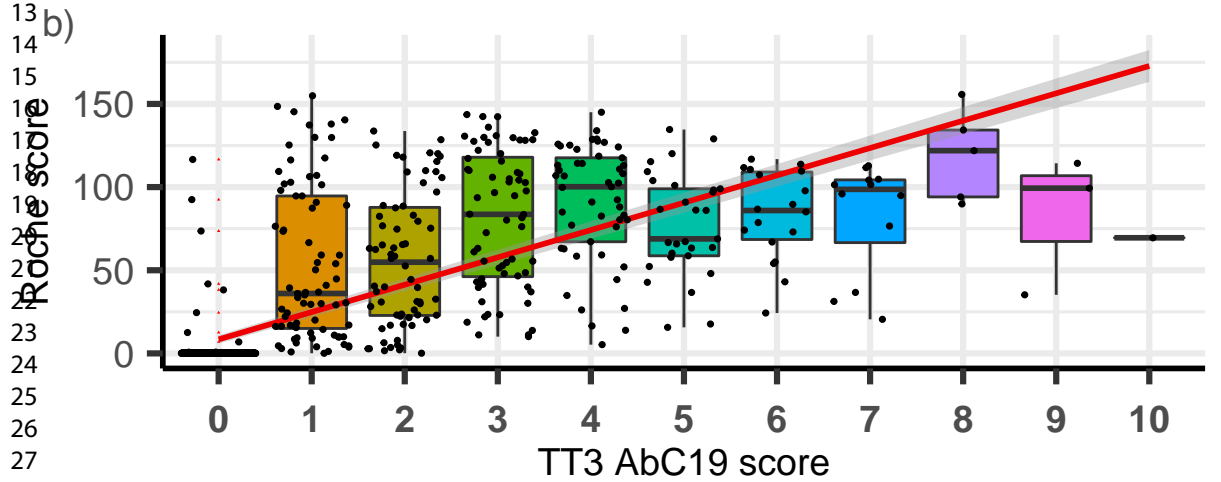
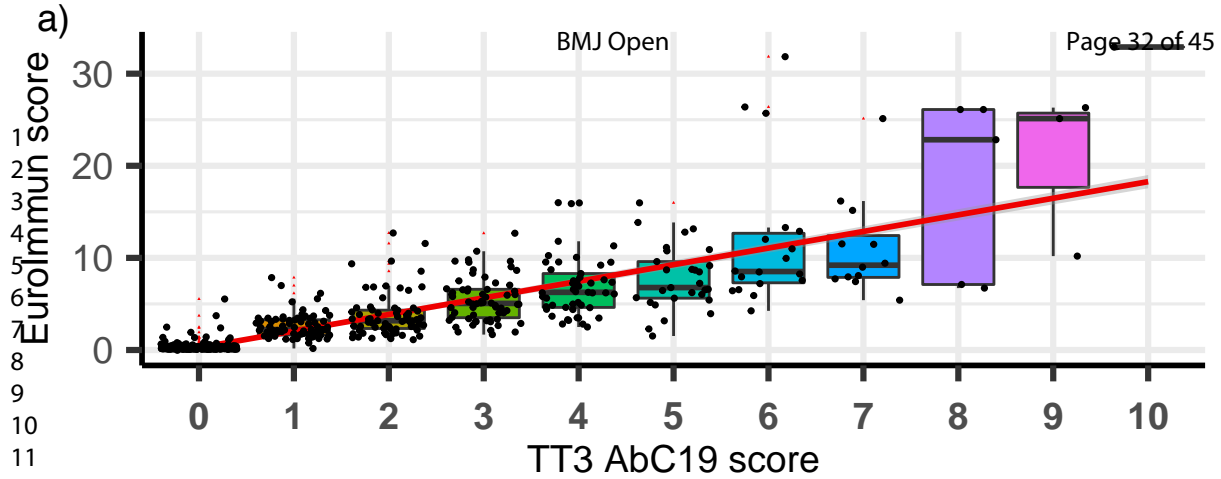
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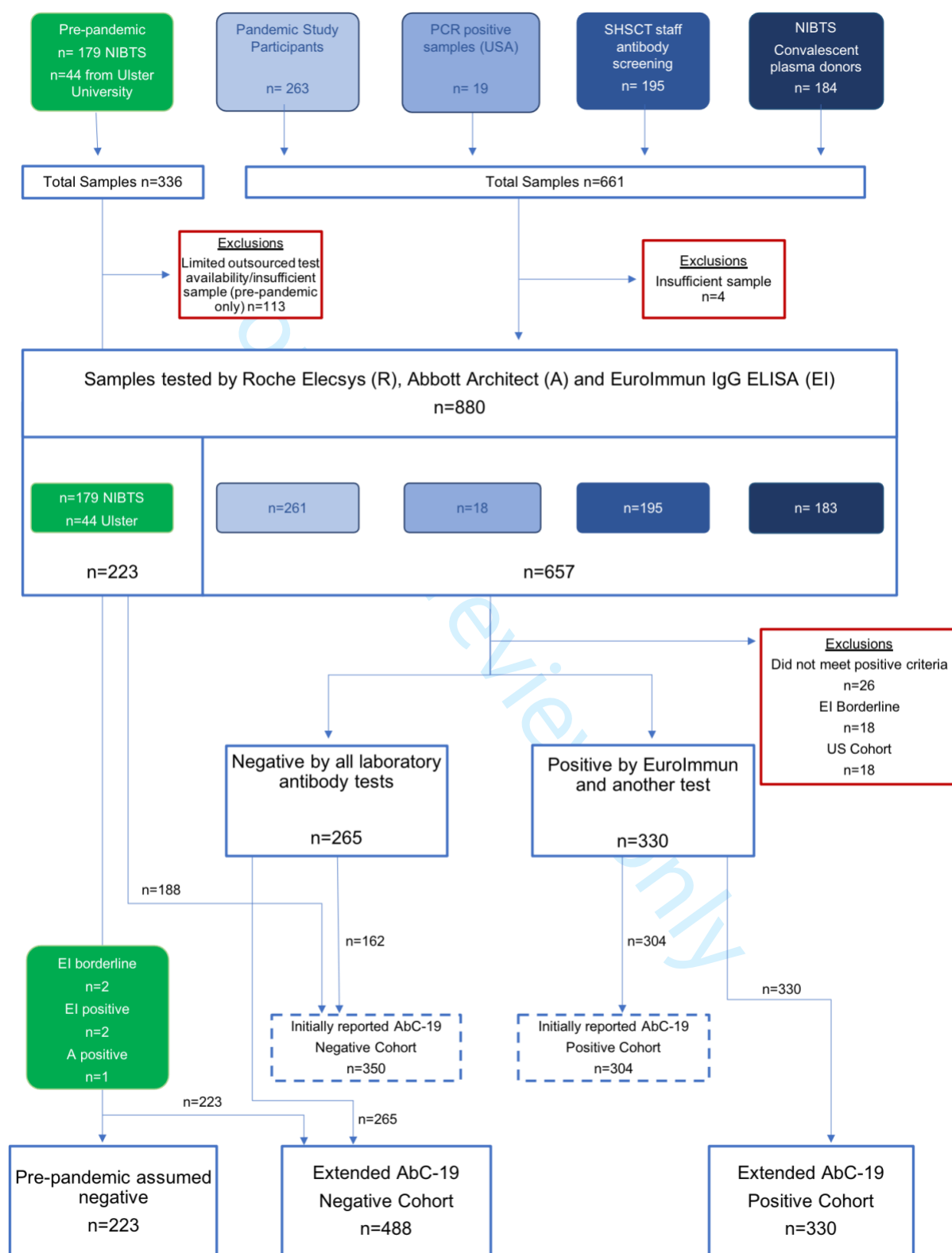
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## Supplementary Materials

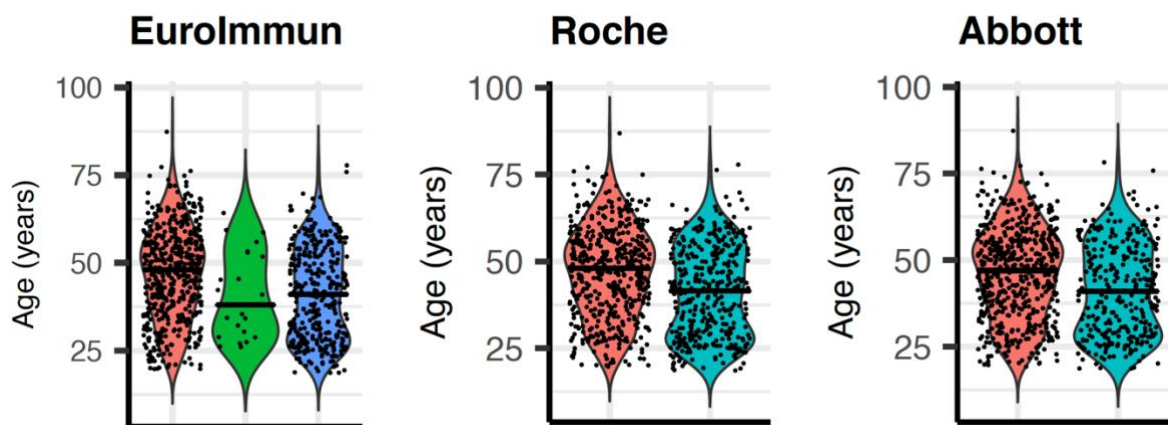


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3 **Figure S1: Flow of participant plasma samples through the study.**  
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5 All available samples from participants within each cohort, and the included and  
6 excluded samples at all stages. Freeze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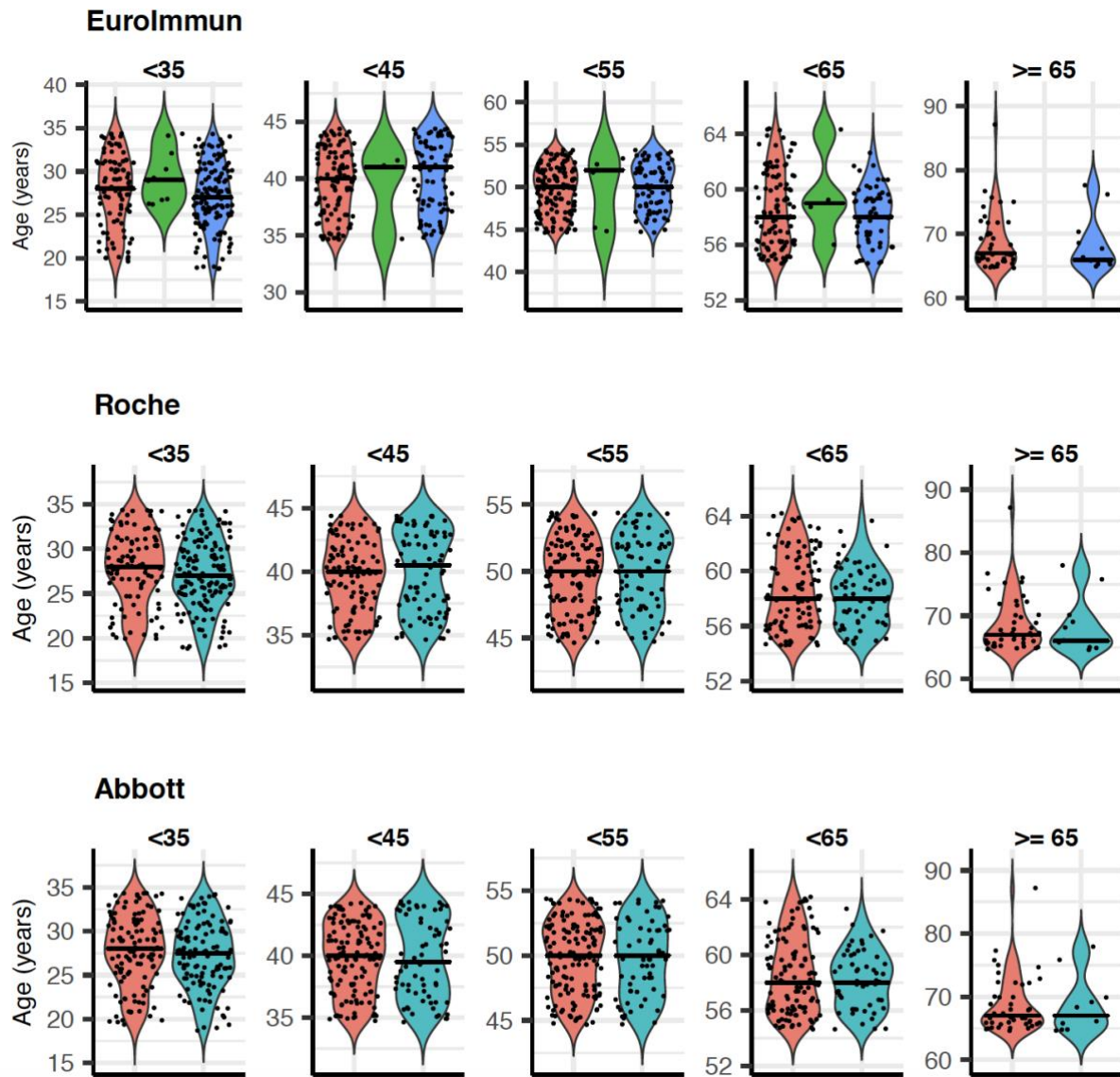


**Figure S2: Visual Score card for quantitative interpretation of AbC-19 LFIA test bands.** A scale of 0 (not pictured, negative-no test line visible) to 10 (positive-strongest test line). Any LFIA scoring 1 or above was classified as positive.



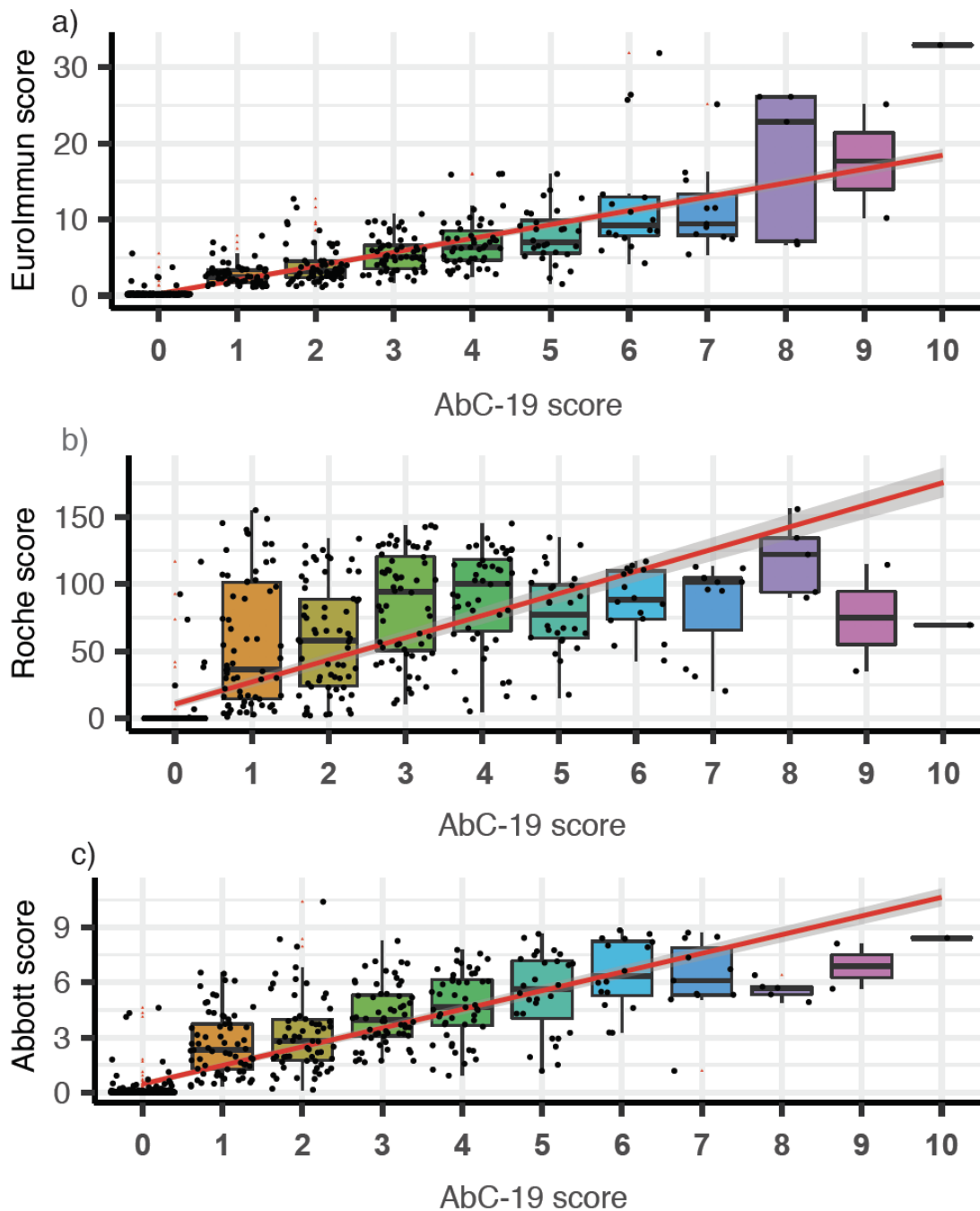
**Figure S3: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.**

The above graphs allow comparison of the distributions and probability density of ages for EuroImmune, 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.**

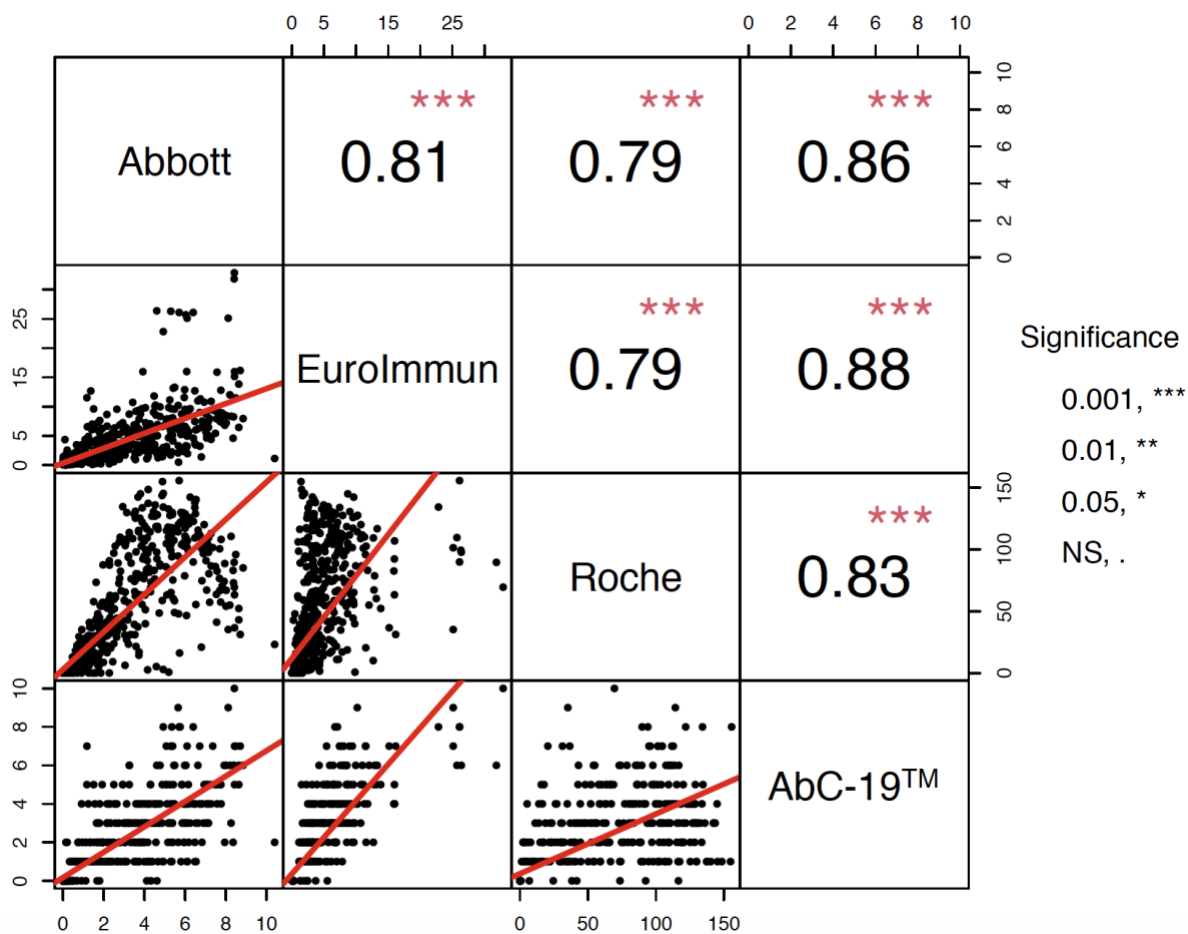
The above figure presents graphs for each immunoassay (EuroImmun, Roche and Abbott) with the corresponding age groups <35 years, <45 years, <55 years, <65 years and  $\geq 65$  years. The red violin plots represent the negative results, the green violin plot represents the borderline EuroImmun results,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



Linear fit line with 95% CI

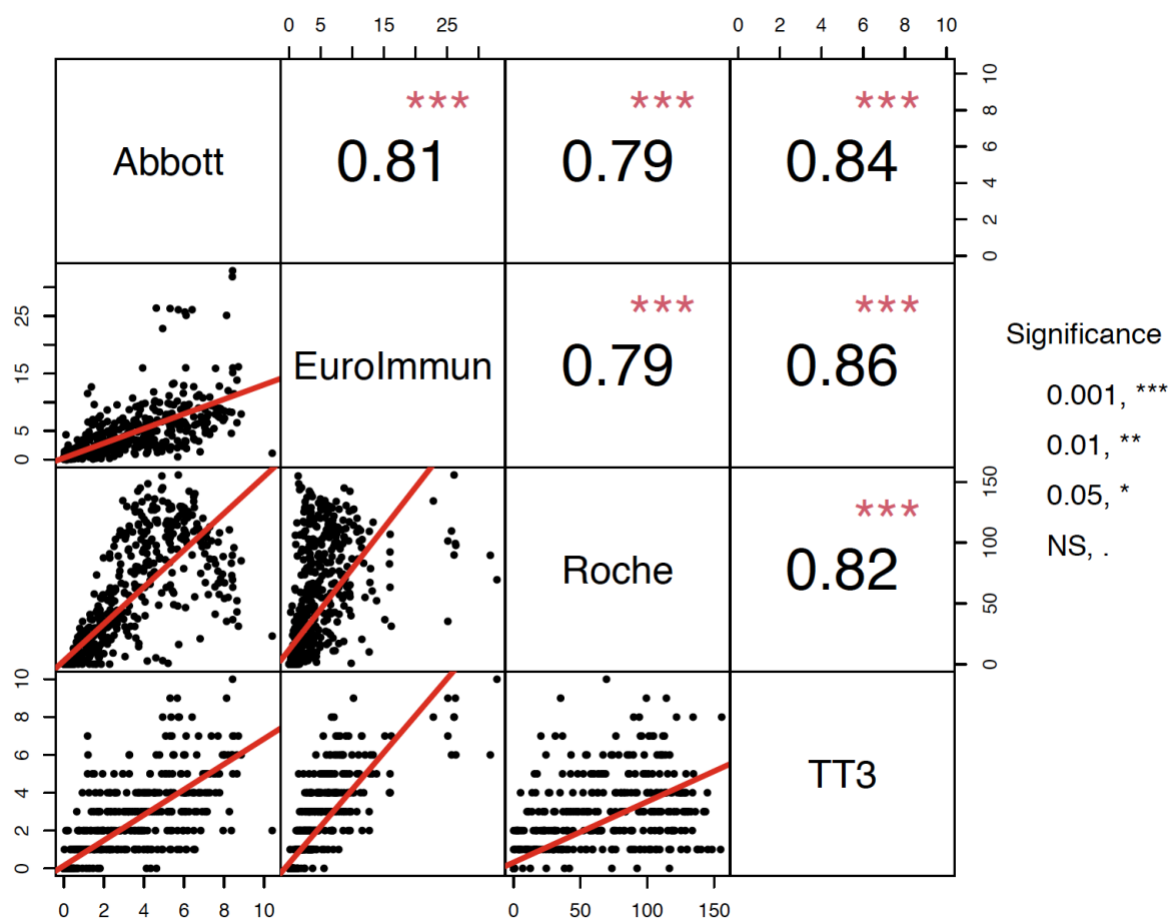
**Figure S5: AbC-19 initially reported cohort n=654 correlation to a) EuroImmun b) Roche and c) Abbott scores.** Box plots overlaid on scatter plot, comparing AbC-19 test scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line of best fit with 95% confidence interval shaded in grey. Black bars indicate median, within IQR (interquartile range) boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on  $1.5 \times$  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
<b>EuroImmun ELISA</b>	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
<b>Roche Elecsys immunoassay</b>	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
<b>Abbott Architect SARS-CoV-2</b>	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
<b>AbC-19</b>	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%)
Eurolmmun	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** LFIA 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

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3 determined negative,  $\geq 0.8$  to  $<1.1$  borderline and  $\geq 1.1$  positive. For a portion of  
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5 samples provided by NIBTS, EuroImmune IgG assay data was provided to researchers  
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7 by NIBTS.  
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12 Roche Elecsys immunoassay (Roche Diagnostics, kit 09203079190) was carried out  
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14 according to manufacturer's instructions on the Roche cobas e601 (C6000 line) or  
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16 e801 (C8000 line) analysers. The analyser automatically calculates the cut-off based  
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18 on the measurement of ACOV2 Cal1 (negative) and ACOV2 Cal2 (positive). The  
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20 result of a sample is given either as reactive or non-reactive as well as in the form of  
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22 a cut-off index (COI; signal sample/cut-off). A score of  $<1.0$  is determined negative,  
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24 while a score  $\geq 1.0$  is positive.  
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31 Abbott Architect SARS-CoV-2 immunoassay was carried out according to  
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33 manufacturer's instructions on the Abbott Architect i2000SR analyser (Abbott,  
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35 kit 18115FN00, calibrator kit 17412FN00, Control kit 17531FN00). The external  
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37 control is entered into a Quality Monitor programme and must be within 3 standard  
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39 deviations of the mean (cumulative; External control NIBSC QCRSARSCoV-2QC1 Lot  
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41 20/B764-01). Results are reported by dividing the sample result by the calibrator result.  
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43 The result unit for the SARS-CoV-2 IgG assay is Index (Sample/Calibrator). A ratio of  
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45  $< 1.4$  is determined negative and  $\geq 1.4$  is determined positive.  
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#### 51 *Analytical specificity and sensitivity assessment*

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53 Four virology samples (H5N1 influenza serology 7/150, RSV serology 16/284,  
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55 Influenza B 9/222 and Bordetella Pertusis 89/530) were obtained from NIBSC  
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57 (National Institute for Biological Standards, Herts, UK). An additional 30 serology  
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3 samples from known virus infections were a kind gift from Sugentech, Seoul, Korea.  
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5 15 of these virology samples were obtained from Trina (Trina Bioreactives AG,  
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7 Switzerland) from 5 different individuals per virus (Influenza A IgG, Influenza B IgG  
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9 and RSV IgG). A further 15 of these virology samples were obtained from AbBaltris,  
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11 Kent from 5 different individuals per virus (Haemophilus Influenza IgG, Seasonal  
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13 Coronavirus NL63 and 229E Seasonal Coronavirus). All these serology samples  
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15 alongside a panel of 6 external standard research reagents (Table S4; NIBSC; Cat:  
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17 20/118 and 20/130) were assessed on the AbC-19 LFIA to confirm analytical  
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19 specificity and sensitivity.  
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Section & Topic	No	Item	Reported on page #
<b>TITLE OR ABSTRACT</b>			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
<b>ABSTRACT</b>			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
<b>INTRODUCTION</b>			
	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
<b>METHODS</b>			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
<i>Participants</i>	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
<i>Test methods</i>	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
<i>Analysis</i>	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
<b>RESULTS</b>			
<i>Participants</i>	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
<i>Test results</i>	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
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	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
<b>OTHER INFORMATION</b>			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents



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30	Sources of funding and other support; role of funders	18
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# BMJ Open

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

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<b>Primary Subject Heading:</b>	Infectious diseases
<b>Secondary Subject Heading:</b>	Immunology (including allergy)
<b>Keywords:</b>	COVID-19, Molecular diagnostics < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES





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3 1 **Laboratory evaluation of SARS-CoV-2 antibodies: detectable IgG more than 10**  
4 2 **months post infection.**  
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6

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28 22

1  
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3 **36 Abstract**

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5 *37 Objective*

6  
7 38 To evaluate the dynamics and longevity of the humoral immune response to SARS-  
8  
9 39 CoV-2 infection and assess the performance of professional use of  
10  
11 40 the UK-RTC AbC-19 Rapid Test lateral flow immunoassay (LFIA) for the target  
12  
13 41 condition of SARS-CoV-2 spike protein IgG antibodies.

14 *42 Design*

15  
16 43 Nationwide serological study.

17 *44 Setting*

18  
19 45 Northern Ireland, UK, May 2020- February 2021.

20  
21 *46 Participants*

22  
23 47 Plasma samples were collected from a diverse cohort of individuals from the general  
24  
25 48 public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood  
26  
27 49 donations and research studies (n=223) and through a convalescent plasma  
28  
29 50 program (n=183). Plasma donors (n=101) were followed with sequential samples  
30  
31 51 over 11 months post symptom onset.

32  
33 *52 Main Outcome Measures*

34  
35 53 SARS-CoV-2 antibody levels in plasma samples using Roche Elecsys Anti-SARS-  
36  
37 54 CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2  
38  
39 55 ELISA immunoassays over time. UK-RTC AbC-19 LFIA sensitivity and specificity,  
40  
41 56 estimated using a three-reference standard system to establish a characterised  
42  
43 57 panel of 330 positive and 488 negative SARS-CoV-2 IgG samples.

44  
45 *58 Results*

46  
47 59 We detected persistence of SARS-CoV-2 IgG antibodies for up to 10 months post  
48  
49 60 infection, across a minimum of two laboratory immunoassays. On the known positive  
50  
51 61 cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58%  
52  
53 62 (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-  
54  
55 63 99.95%).

56  
57 *64 Conclusions*

58  
59 65 Through comprehensive analysis of a cohort of pre-pandemic and pandemic  
60  
61 66 individuals, we show detectable levels of IgG antibodies, lasting over 46 weeks when  
62  
63 67 assessed by EuroImmun ELISA, providing insight to antibody levels at later time points  
64  
65 68 post-infection. We show good laboratory validation performance metrics for the AbC-

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3 69 19 rapid test for SARS-CoV-2 spike protein IgG antibody detection in a laboratory-  
4 based setting.  
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## 8 9 72 **Strengths and Limitations**

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11 73 Strength - This paper describes a non-clinical laboratory evaluation and comparison  
12 of the ability of three different immunoassays to detect SARS-CoV-2 antibodies in the  
13 same samples, detecting different subtypes of antibodies against different targets of  
14 the viral antigenic repertoire, that does not rely on PCR-positivity as definition of  
15 expected test outcome, to provide a panel of known antibody positive and antibody  
16 negative serology for evaluation of newly developed immunoassays.  
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26  
27 80 Strength - This study demonstrates AbC-19 lateral flow point of care detection of IgG  
28 antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the antibodies made  
29 in response to the vaccines used globally, in a large cohort of subjects, more than 10  
30 months post infection, across a broad age range (18-78 years). Robust antibody  
31 responses were observed in all age groups tested, including over-65s, who are most  
32 at risk of severe COVID-19 symptoms, and were prioritised in the UK-wide mass  
33 vaccination programme.  
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45 88 Strength - This study shows excellent correlation between approved laboratory-based  
46 assays and the AbC-19 lateral flow point of care lateral flow test for the detection of  
47 SARS-CoV-2 antibodies in characterised cohorts of known positive and negative  
48 plasma samples in an evaluation conducted according to MHRA guidelines during a  
49 pandemic.  
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3 93 Strength- Longitudinal data detecting IgG antibodies more than 10 months from  
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5 94 infection was collected as sequential samples over time through a convalescent  
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8 95 plasma donation program.  
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10 96  
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12 97 Limitation- This study was conducted in a standardised setting with very experienced  
13  
14 98 users on plasma characterised as positive or negative for the presence of antibodies  
15  
16 99 using a reference standard, alongside one other assay which may introduce a possible  
17  
18 100 spectrum bias. The laboratory setting may not reflect the true performance metrics of  
19  
20 101 the assay evaluated when translated to real life settings, using finger prick blood  
21  
22 102 samples and in which pre-test probability would impact greatly on positive and  
23  
24 103 negative predictive values.  
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### 30 105 **Keywords**

31  
32 106 SARS-CoV-2, COVID-19, immunoassay, LFIA, pandemic, antibody assay  
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35 107

### 36 37 108 **Introduction**

38  
39 109 The World Health Organization declared a pandemic in March 2020 due to severe  
40  
41 110 acute respiratory syndrome coronavirus-2 (SARS-CoV-2), identified late 2019 in  
42  
43 111 Wuhan, China, causing Coronavirus Disease 2019 (COVID-19) disease (1,2).  
44  
45

46 112 A global race ensued to develop diagnostic assays, with the most common being viral  
47  
48 113 RNA detection (RT-qPCR assays), to detect acute infection(3). RT-qPCR assays are  
49  
50 114 labour and reagent intensive, limited by a short temporal window for positive diagnosis,  
51  
52 115 and exhibit potential for false negative results (4). Evidence suggests sensitivity of RT-  
53  
54 116 qPCR can be as low as 70% (5). False positive rates between 0.8- 4.0% have been  
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56 117 reported in the UK and are dependent on the Ct values accepted as indicating  
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3 118 infection, the number of SARS-CoV-2 genes analysed, and the proportion of  
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5 119 asymptomatic individuals tested (6,7). Lockdown measures and “flattening the curve”  
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8 120 strategies in the UK meant many infected individuals were instructed to self-isolate  
9  
10 121 and were not offered a diagnostic RT-qPCR, with much of the testing limited to patients  
11  
12 122 admitted to hospital, who perhaps reflect a more severely infected cohort.  
13  
14 123 Consequently, a potentially large number of cases were unconfirmed or undetected  
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16  
17 124 (8).

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19  
20 125 The ability to accurately detect SARS-CoV-2 specific antibodies, which develop after  
21  
22 126 an immune response is evoked, is vital for building biobanks of convalescent sera for  
23  
24 127 treatment, monitoring immune response to infection alongside surveillance studies  
25  
26 128 and assessing responses to vaccination programmes.

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28  
29 129 Commercial serology immunoassays are mostly laboratory-based and measure IgG  
30  
31 130 antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIA),  
32  
33 131 require a finger prick blood sample and can be used at point-of-care (POC) or in the  
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35 132 home; particularly important in the context of lockdown enforcement during the  
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37 133 pandemic. A limited number of laboratory-based chemiluminescence immunoassays  
38  
39 134 are approved for use in the UK including the Roche Elecsys Anti-SARS-CoV-2  
40  
41 135 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region (Roche  
42  
43 136 Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay against the  
44  
45 137 same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

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47  
48 138 The complexities of the humoral immune response to SARS-CoV-2 is a much-  
49  
50 139 debated topic. In a US study, approximately one in 16 individuals lacked detectable  
51  
52 140 IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR  
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54 141 confirmed infection (9). Patients who remain asymptomatic may mount a humoral  
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56 142 immune response which is short-lived, with detectable levels of antibody falling

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3 143 rapidly (10). This, alongside the lack of RT-PCR test availability across the UK has  
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5 144 hindered development of well characterised gold standard serology test for IgG  
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7 145 antibodies to SARS-CoV-2.  
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9  
10 146 Herein, we describe the use of Roche and Abbott commercial immunoassays, as well  
11  
12 147 as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike  
13  
14 148 (antigenic) protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-  
15  
16 149 pandemic and pandemic COVID-19 blood samples (n=880) from within Northern  
17  
18 150 Ireland and report on longevity of IgG antibodies detected. Furthermore, we follow IgG  
19  
20 151 antibody levels in convalescent plasma donors (n=101 individuals) for up to 11  
21  
22 152 months. Currently, there is no gold standard assay for comparison, therefore we aimed  
23  
24 153 to establish a reference based on a positive COVID-19 antibody status. We present  
25  
26 154 results of a laboratory evaluation of the UK-RTC AbC-19 with a target condition of  
27  
28 155 antibodies against a cohort of 330 known IgG antibody positive samples according to  
29  
30 156 this 'positive by two' system and 488 negative samples (223 pre-pandemic assumed  
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32 157 negative and 265 known negative) for IgG to SARS-CoV-2.  
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## 159 **Methods**

### 161 **Participant samples**

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

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3 168 The first 800 respondents who expressed interest were provided with an online patient  
4  
5 169 information sheet, consent form and health questionnaire and invited to register to  
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7 170 attend a clinic. Participants were eligible for the study if they were over 18 years of  
8  
9 171 age. Exclusion criteria included anyone with a blood disorder or contraindication to  
10  
11 172 giving a blood sample, or anyone currently exhibiting symptoms of COVID-19. To  
12  
13 173 enrich the cohort for samples potentially positive for SARS-CoV-2 IgG antibody,  
14  
15 174 further participants were invited if they had previously tested PCR positive or had the  
16  
17 175 distinctive symptom of loss of taste and smell. Blood sampling clinics were held at  
18  
19 176 locations around Northern Ireland between May and July 2020 resulting in collection  
20  
21 177 of 263 10ml EDTA plasma samples from 263 separate study participants. Additional  
22  
23 178 anonymised plasma samples were obtained from Southern Health and Social Care  
24  
25 179 Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood Transfusion  
26  
27 180 Service (NIBTS, n=184) through convalescent plasma programs. NIBTS convalescent  
28  
29 181 plasma samples continued to be collected throughout 2020-early 2021, with a total of  
30  
31 182 n=897 from n=676 individuals, including n=183 samples from the cross-sectional  
32  
33 183 cohort. Individuals from this program with a positive RT-PCR result and EuroImmun  
34  
35 184 starting value >6 were sequentially sampled over a period of up to 46 weeks resulting  
36  
37 185 in a cohort of n=101 individuals, n=296 samples (including n=47 individuals from the  
38  
39 186 cross-sectional cohort).  
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49 188 Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster  
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51 189 University ethics committee approved studies with ongoing consent and from NIBTS  
52  
53 190 (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-  
54  
55 191 thaw cycles for all analyses reported within this manuscript.  
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## 193 **Clinical information**

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

200

## 201 **Laboratory-based immunoassays**

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

204

## 205 **UK-RTC AbC-19 LFIA**

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

214

## 215 **Statistical analysis**

216 As per Daniel (11) a minimum sample size based on prevalence can be calculated

217 using the following formula:  $n = \frac{Z^2 P(1 - P)}{d^2}$ , where n = sample size, Z = Z statistic for a

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2  
3 218 chosen level of confidence,  $P$  = estimated prevalence, and  $d$  = precision. Assuming  
4  
5 219 a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the  
6  
7 220 required sample size at 99% confidence ( $Z = 2.58$ ) to be 240 individuals. If the true  
8  
9 221 prevalence is lower, 5%, the estimated required sample size given a precision of  
10  
11 222 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200  
12  
13 223 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody  
14  
15 224 immunoassays(12).

16  
17 225 Statistical analysis was conducted in in R v 4.0.2(13). To assess discordance between  
18  
19 226 test results, data was first filtered to include individuals with an Abbott test result in the  
20  
21 227 range  $\geq 0.25$  &  $\leq 1.4$ , with a 2 x 2 contingency table produced that comprised all  
22  
23 228 possible combinations of [concordant|discordant] test results [within|outside of] this  
24  
25 229 range. A p-value was derived via a Pearson  $\chi^2$  test after 2000 p-value simulations via  
26  
27 230 the stats package.

28  
29 231 AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc  
30  
31 232 Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To  
32  
33 233 compare test result (Positive|Negative) to age, a binary logistic regression model was  
34  
35 234 produced with test result as outcome – a p-value was then derived via  $\chi^2$  ANOVA. To  
36  
37 235 compare time against test result (encoded continuously), a linear regression was  
38  
39 236 performed. We calculated median per time-period and then converted these to log  
40  
41 237 [base 2] ratios against the positivity cut-off for each assay. All plots were generated  
42  
43 238 via ggplot2 or custom functions using base R(14).

44  
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## 46 47 240 **Results**

48  
49 241 We analysed samples from a mixed cohort of individuals from the general public  
50  
51 242 (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations  
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3 243 and research studies (n=223) and through a convalescent plasma program (n=183).  
4  
5 244 Antibody levels in plasma from these 880 individuals were assessed using the three  
6  
7 245 SARS-CoV-2 immunoassays; EuroImmun IgG, Roche Elecsys IgG/IgM/IgA and  
8  
9 246 Abbott Architect IgG (Table S1, Figure S3). This included a cohort of 223 pre-  
10  
11 247 pandemic plasma samples collected and stored during 2017 to end of May 2019 to  
12  
13 248 determine assay specificity. Of the 657 participants whose samples were collected  
14  
15 249 during the pandemic, 267 (40.64%) previously tested RT-PCR positive with a range of  
16  
17 250 7-173 days since diagnosis. A total of 225 participants gave time since self-reported  
18  
19 251 COVID-19 symptoms, with a range of 5-233 days from symptom onset, whilst 195 had  
20  
21 252 no symptom or PCR data available. Samples collected in 2020 (n=657) ranged from  
22  
23 253 19-78 years of age with a median (IQR) of 43 years ( $\pm 22$ ), and n=454 were female  
24  
25 254 and n=200 male (n=3, not disclosed). Pre-pandemic samples (n=223) ranged from 20-  
26  
27 255 87 years of age with median (IQR) of 50 years ( $\pm 20$ ) and consisted of n=88 female  
28  
29 256 and n=135 male.

### 35 36 257 *Laboratory based antibody immunoassays*

37  
38 258 A positive result for antibody on one or more of the three laboratory immunoassays  
39  
40 259 was recorded for 385/657 (58.6%) participants who provided a sample during the  
41  
42 260 pandemic. By EuroImmun ELISA, 346 were positive, 20 borderline and 291 were  
43  
44 261 negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott  
45  
46 262 determined 310 positive and 347 negative (Table S2, Figure S3). The median age  
47  
48 263 across all age groups combined was lower for participants testing positive across each  
49  
50 264 of the immunoassays (median [sd] for positive versus negative, respectively:  
51  
52 265 EuroImmun, 41 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41  
53  
54 266 [13.18] vs 47 [13.09]). (Figure S4,  $p < 0.0001$ ). When segregated by age group,  
55  
56 267 however, differences were less apparent in certain groups (Figure S5). Excluding the  
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3 268 pre-pandemic cohort, this gap reduced but remained statistically significant  
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5 269 EuroImm, 41 [13.18] vs 45 [12.49]; Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41  
6  
7 [13.26] vs 44 [12.63]) ( $p < 0.01$ ) (median [sd] for positive versus negative). Of note, out  
8  
9 of 267 individuals with a previous positive RT-PCR result for SARS-CoV-2 viral RNA,  
10  
11 271 14 (5.2%, Figure S3a) did not show detectable antibodies by any of the three  
12  
13 272 immunoassays, with no association found with age, gender or time between test and  
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15 273 blood draw (data not shown).  
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20 275 The three commercial laboratory immunoassays provide a ratio value that increases  
21  
22 276 with IgG antibody titre. When correlation between these values is assessed, good  
23  
24 277 overall agreement is observed between the three immunoassays (Figure 1, Figure  
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26 278 S5). As highlighted by Rosadas *et al.*, we also see significant disagreement in the  
27  
28 279 Abbott 0.25-1.4 range when compared to EuroImm and Roche (Figure 1a,b; chi-  
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30 square p-values: EuroImm vs Abbott,  $p < 0.001$ ; Roche vs Abbott,  $p < 0.001$ )(15).  
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### 35 36 37 282 *Duration of humoral response to SARS-CoV-2*

38  
39 283 In a cross-sectional analysis of antibodies over time, we found IgG antibodies could  
40  
41 284 still be detected in individuals (excluding pre-pandemic) across all three  
42  
43 285 immunoassays used up to week 20 (day 140) (Figure 2). We note a statistically  
44  
45 286 significant decrease in signal with respect to time across each assay (p-value  
46  
47 [estimate slope]): EuroImm,  $p = 0.028$ [-0.823]; Roche,  $p = 0.002$  [-0.125]; Abbott,  
48  
49 287  $p < 0.0001$  [-3.673]. These remained statistically significant after adjustment for age.  
50  
51 288  
52  
53 289 Antibody levels (expressed as a ratio of median result per timepoint divided by  
54  
55 290 positivity cut off; Table 1) peaked at Week 1-2 for EuroImm (1.33) and Abbott  
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57 291 (1.64), though reached highest levels at Week 8-12 when measured by Roche  
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59 292 (5.45). By week 21-24, median score for all tests had dropped below the positivity



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3 293 cut off, though a small number of samples remained above the positive cut off at  
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5 294 these later timepoints (Figure 2).  
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7 295 Samples from the NIBTS convalescent plasma program continued to be collected  
8  
9 296 throughout 2020-early 2021. A total of n= 897 samples from n=676 individuals were  
10  
11 297 collected, 744/883 tested by EuroImmun were positive (>1.1, with values range of  
12  
13 298 0.051-34.361), 556/749 tested by Abbott were positive (>1.4, with values ranging  
14  
15 299 from 0.01-8.85). Individuals with a positive RT-PCR result and a EuroImmun result  
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17 300 >6 were sequentially sampled (with median 3, range 2-9 samples per individual) and  
18  
19 301 analysed by both EuroImmun (n=101 individuals), and Abbott immunoassays (n=75  
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21 302 individuals). Median age (IQR) for this cohort is 51 years ( $\pm 21$ ) with a range from 18-  
22  
23 303 70 years and n=27 female, n=74 male. Longitudinal analysis shows persistence of  
24  
25 304 detectable IgG antibodies until up to 302 days (43 weeks) by Abbott immunoassay  
26  
27 305 (at which point this assay was discontinued at NIBTS) and 323 days (46 weeks) by  
28  
29 306 EuroImmun ELISA, with a gradual decline over time (Figure S6). None of the  
30  
31 307 individuals who were initially positive by Euroimmun SARS-CoV-2 S1 IgG assay  
32  
33 308 dropped to below the EuroImmun positivity threshold (>1.1) over the course of the  
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35 309 follow-up while 26 who were initially positive by Abbott SARS-CoV-2 NP IgG fell  
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37 310 below the Abbott threshold (>1.4).  
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### 311 312 *UK-RTC AbC-19*

313 Using the commercial immunoassays described we established a well characterised  
314 serology sample set of 'known positive' and 'known negative' for IgG antibodies to  
315 SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.  
316 AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore  
317 required all samples to be positive by the EuroImmun SARS-CoV-2 IgG ELISA, which



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3 318 likewise detects antibodies against the S1 domain (16). To develop this characterised  
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5 319 cohort, samples were also required to be positive by a second immunoassay (Roche  
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7 320 or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG  
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9 321 antibody, we assessed 350 plasma samples from participants classed as 'known  
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11 322 negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from  
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13 323 individuals confirmed to be negative across all three laboratory assays (Roche,  
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15 324 EuroImmun, Abbott). Using these positive n=304 and negative n=350 antibody  
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17 325 cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of  
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19 326 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the  
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21 327 AbC-19 LFIA (Table 2).

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26 328 Given a recent report of lower specificity in the AbC-19 LFIA (17) and the possibility  
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28 329 of introducing sample bias, we revised our inclusion criteria for the negative cohort.  
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30 330 For the pre-pandemic cohort, we included samples from all 223 individuals,  
31  
32 331 regardless of results on other laboratory immunoassays. When this assumed  
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34 332 negative pre-pandemic cohort was used for laboratory evaluation for target condition  
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36 333 of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 2). We  
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38 334 expanded the negative cohort to include all samples that matched our criteria  
39  
40 335 (samples collected during the pandemic to be negative by all three laboratory assays  
41  
42 336 and all pre-pandemic samples regardless of other immunoassay results). The  
43  
44 337 specificity observed on this extended negative cohort of 488 samples was 99.59%  
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46 338 (98.53% to 99.95%, Table 2). For sensitivity analysis on a positive cohort (samples  
47  
48 339 positive by EuroImmun and one other test), we were able to analyse all samples  
49  
50 340 previously untested due to limited testing capacity and tested a positive cohort of 330  
51  
52 341 samples giving a sensitivity of 97.58% (95.28% to 98.95%, Table 2). When we  
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54 342 sorted samples analysed in both negative (n=488) and positive cohorts (n=330) by  
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3 343 RT-PCR status and assessed AbC-19 LFIA sensitivity by including only those that  
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5 344 were RT-PCR positive (n=227), the test showed a sensitivity of 92.07% (87.76%-  
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7 345 95.23%, Table S3). However, of the n=18 RT-PCR positive individuals negative for  
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9 346 IgG antibodies by AbC-19, n=12 showed no detectable antibodies by all three  
10  
11 347 laboratory assays (EuroImmun, Roche or Abbott), suggesting that antibodies are not  
12  
13 348 present in those samples.  
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19 350 When used as intended by the public, the AbC-19 LFIA provides binary  
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21 351 positive/negative results. However, when assessing LFIA in the laboratory, each test  
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23 352 line was scored against a scorecard by three independent researchers (0 negative, 1-  
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25 353 10 positive; Figure S2). When compared to quantitative outputs from the Abbott,  
26  
27 354 EuroImmun and Roche assays, the AbC-19 LFIA shows good correlation (Abbott  
28  
29 355  $r=0.84$  [ $p<0.001$ ]; EuroImmun  $r=0.86$  [ $p<0.001$ ]; Roche  $r=0.82$  [ $p<0.001$ ]; Figure 3,  
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31 356 Figure S7-Figure S9).  
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### 358 *Analytical specificity and sensitivity of AbC-19 LFIA*

39  
40 359 We observed no cross-reactivity across samples with known H5N1 influenza,  
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42 360 Respiratory syncytial virus, Influenza A, Influenza B, Bordetella Pertussis,  
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44 361 Haemophilus Influenzae, Seasonal coronavirus NL63 and 229E on the AbC-19 LFIA  
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46 362 (n=34 samples, n=8 distinct respiratory viruses; Table S4). Against a panel of external  
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48 363 reference SARS-CoV-2 serology samples, the AbC-19 LFIA detected antibodies with  
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50 364 scores commensurate to the EuroImmun ELISA scores (Figure S10, Table S5).  
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## 56 366 **Discussion**

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3 367 Serological antibody immunoassays are an important tool in helping combat the  
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5 368 SARS-CoV-2 pandemic. The duration of the humoral immune response is of  
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8 369 particular importance, to inform an individual's protection following both natural  
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10 370 infection and vaccination. Using a large cohort of individuals across a wide age  
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12 371 range (18-78), we assessed antibody levels across up to three laboratory  
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14 372 immunoassays perform a cross-sectional and longitudinal analysis over time. Our  
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16 373 results show strong correlation between all three immunoassays, with shortcomings  
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18 374 in the Abbott system output 0.25-1.4 range, as described previously, suggesting an  
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20 375 overestimated positive cut-off (Figure 1) (15).  
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26 377 Longitudinal studies on SARS-CoV-1 convalescent patients suggests that detectable  
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28 378 IgG can still be present as long as 2 years after infection (18). There are conflicting  
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30 379 reports of the longevity of the humoral response to SARS-CoV-2 infection which  
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32 380 differ in the make-up of the cohort studied, the assays used, and the length of time  
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34 381 since symptom onset. To our knowledge, this study represents the longest follow-up  
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36 382 period with detection of IgG antibodies to both spike and nucleocapsid protein more  
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38 383 than 10 months after RT PCR positive status (and beyond in a small number of  
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40 384 samples, Figure 2, Figure S6). In this study, samples were collected through a  
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42 385 convalescent plasma program (Figure S6), with individuals selected for sequential  
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44 386 plasma donation based on an initial high EuroImmun assay score. In contrast to the  
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46 387 time series analysis of healthcare workers recruited prospectively by Manisty *et al.*,  
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48 388 we observed no cases where Euroimmun ELISA-measured anti-Spike antibody  
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50 389 levels fell below threshold, whilst a large number of Abbott measured anti-  
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52 390 Nucleocapsid antibody levels dropped below the positivity threshold (34.7% 26/75).  
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54 391 However, this may be an overestimate given the shortcomings of the Abbott assay  
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3 392 described above (Figure 1) (19). In a similar longitudinal study of 51 symptomatic  
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5 393 participants, Dan *et al.* estimated that half-life ( $t_{1/2}$ ) for IgG-Spike (103 days) was  
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7 394 longer than that for IgG-Nucleocapsid (68 days), although with a considerable  
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9 395 overlap of 95% confidence intervals (20).

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11  
12 396 In our more diverse cross-sectional cohort, we also note a statistically significant  
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14 397 decline over time but levels remain detectable at 140 days (Figure 2). We note that  
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16 398 IgG levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as  
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18 399 Week 8-12 from first symptoms or a viral RNA RT-PCR positive result, though this  
19  
20 400 may be an artefact of lower number of participants at earlier timepoints (Table 1).  
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22 401 Robust antibody responses are produced in our cohorts across a wide age range  
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24 402 (18-78 years old, Figure 2, Figure S6). We detect a slightly but significantly lower  
25  
26 403 median age of participants testing positive (Figure S4); however, this is likely be due  
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28 404 to cohort characteristics and not a true reflection of the population or indication of  
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30 405 test performance.  
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407 A difficulty faced in validation of antibody diagnostic assays has been access to  
408 samples with known SARS-CoV-2 antibody status. As previously described, there is  
409 no clear gold standard reference against which to assess SARS-CoV-2  
410 immunoassays. A positive RT-PCR test has been used previously to indicate previous  
411 (COVID-19) SARS-CoV-2 infection, though this approach is limited by a high rate of  
412 false negatives and positives in RT-PCR testing, failure in some cases to develop IgG  
413 antibodies (sero-silence or lack of antibody against the same antigenic component of  
414 the virus as the immunoassay uses as a capture antigen) and the lack of RT-PCR  
415 testing availability early in the pandemic (3,5,21). SARS-CoV-2 IgG antibodies were  
416 undetectable in 14 of 267 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA

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3 417 positive participants in this study. It is unclear if this is due to insufficient/absent  
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5 418 antibody production in these individuals at the time the sample was taken, or due to a  
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7 419 false positive PCR result which may occur in the UK at a rate between 0.8- 4.0% (6).  
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9 420 Self-assessment of symptoms for COVID-19 (disease) is a poor indicator of previous  
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11 421 infection, even amongst healthcare workers (22). Additionally, the kinetics of a SARS-  
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13 422 CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus  
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15 423 with time, contributing to false negative RT-PCR test results for individuals who may  
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17 424 be late to present for virus detection tests (5,23).  
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24 425  
25 426 To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-  
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27 427 CoV-2 antibody in a laboratory evaluation, we developed a reference standard for  
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29 428 SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar  
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31 429 approach was used in a recent seroprevalence study in Iceland, whereby two positive  
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33 430 antibody results were required to determine a participant sample as positive for SARS-  
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35 431 CoV-2 antibody (21). Our evaluation of performance metrics for the UK-RTC AbC-19  
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37 432 LFIA to detect antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59%  
38  
39 433 specificity. In an evaluation of the AbC-19 tests, Mulchandani *et al.* observed a  
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41 434 specificity of 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report  
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43 435 a sensitivity of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a  
44  
45 436 previous RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys  
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47 437 antibody test, which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid  
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49 438 portion of SARS-CoV-2 (22). In RT-PCR positive individuals from our cohorts, the  
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51 439 AbC-19 test showed a similar sensitivity (92.07%, 87.76%- 95.23%, Table S3).  
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53 440 However, we demonstrate the drawbacks of this approach given that in 12 of 18 AbC-  
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55 441 19 false negatives, none of the four immunoassays used (EuroImmuno, Roche, Abbott  
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3 442 or AbC-19) detected antibodies, suggesting either a false RT-PCR result, a failure to  
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5 443 produce IgG antibodies or sero-reversion before sample collection in these individuals.  
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7 444 Another recent evaluation of the AbC-19 LFIA by Moshe *et al.* determined a sensitivity  
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9 445 of (100% (98.1-100%)) on laboratory sera, using a composite reference standard of  
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11 446 antibody positive by either Spike protein ELISA or hybrid DABA assay and specificity  
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13 447 of 99.8% (98.9-100%) against pre-pandemic samples. However, when AbC-19  
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15 448 performance was analysed on matched finger-prick and serum samples against the  
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17 449 same antibody standard, a lower sensitivity was observed (finger-prick 69% (53.8-  
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19 450 81.3%), serum 92% (80-97.7%)) (24).

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26 452 In our study, strong correlation was observed in quantitative score between results on  
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28 453 all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA  
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30 454 (Figure S8, S9). This is to be expected, given both the AbC-19 LFIA and EuroImmun  
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32 455 ELISA detect IgG antibodies against spike protein. Importantly, for the assessment of  
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34 456 immunity to prior natural infection as well as to immunisation, IgG antibodies against  
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36 457 SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA and AbC-  
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38 458 19 LFIA are known to correlate with neutralizing antibodies, which may confer future  
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40 459 immunity (20,25,26). Previous evaluations of sensitivity and specificity reported by  
41  
42 460 Public Health England (PHE), showed a EuroImmun sensitivity of 72% and specificity  
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44 461 of 99%, Abbott with sensitivity of 92.7% and specificity of 100% and Roche with  
45  
46 462 sensitivity of 83.9% and specificity of 100% (27–29). The PHE analyses for each of  
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48 463 these tests used previous infection (RT-PCR positive status) as a reference standard,  
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50 464 the limitations of which are discussed above.

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3 466 In the use of characterised 'known positive' and 'known negative' cohorts, one  
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5 467 limitation of this study is its potential for spectrum bias, whereby our positive-by-two  
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7 468 reference system may artificially raise the threshold for positive sample inclusion,  
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9 469 possibly resulting in the overestimation of the sensitivity of any test evaluated (30).  
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11 470 However, similar issues have been raised when using previous RT-PCR result or  
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13 471 definitive COVID-19 symptoms as inclusion criteria given these will likely skew a  
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15 472 cohort towards more severe disease, especially given issues of RT-PCR availability  
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17 473 outside of hospital settings during the first wave (5). Importantly, our mixed origin of  
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19 474 samples forming the cohort provides a positive cohort for assessing assay sensitivity  
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21 475 that includes individuals from the general public, healthcare workers and from  
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23 476 convalescent plasma programmes. In the absence of a clear gold standard test, our  
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25 477 system relies on no single test (each with their individual shortcomings) and instead  
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27 478 takes an average of three. Our analysis of specificity on only pre-pandemic individuals  
28  
29 479 (n=223) shows similar specificity (99.55%) to the larger mixed 'known negative cohort'  
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31 480 (n=488, sensitivity 99.59%). We also demonstrate a high level of analytical specificity  
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33 481 of the AbC-19 test with no cross-reactivity against a panel of other respiratory viruses,  
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35 482 including SARS-CoV-1 NL63 and 229E (Table S4).  
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484 Our assessment of the AbC-19 LFIA in a laboratory setting, using characterised  
485 cohorts of known SARS-CoV-2 antibody positive and antibody negative plasma,  
486 shows good performance metrics for its ability to detect SARS-CoV-2 IgG antibodies  
487 following natural infection. We note our use of plasma from venous blood samples, as  
488 opposed to a finger prick blood sample as would be used in rapid testing scenarios  
489 (24). Additionally, when the AbC-19 LFIA was used on our cohort, a number of the  
490 positive results scored low (1/10 using the score card under laboratory conditions,



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3 491 Figure 3), with a faint test band visible to a trained laboratory scientist but perhaps  
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5 492 difficult to identify as positive by individuals performing a single test (Figure S10). This  
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7 493 faint line may be reflective of the longer time from infection for the Northern Ireland  
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9 494 cohort used. If the AbC-19 LFIA is to be used in clinical settings it is important to  
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11 495 determine if all users observe the same results as observed in this laboratory  
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13 496 evaluation.  
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19 498 This assessment of the AbC-19 LFIA does not provide data on how this test will  
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21 499 perform in a seroprevalence screening scenario, but instead provides metrics for the  
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23 500 performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as  
24  
25 501 opposed to previous COVID-19 infection. An important potential use of the AbC-19  
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27 502 LFIA would be in monitoring the immune response to vaccination, with most vaccines  
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29 503 utilising SARS-CoV-2 Spike protein antigens (31).  
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33 504

## 35 505 **Conclusion**

37 506 We present a comprehensive analysis of pre-pandemic and two large pandemic  
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39 507 cohorts (more than 700 individuals) and in a longitudinal analysis showing that IgG  
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41 508 antibodies to SARS-CoV-2 antigens are detectable more than 10 months from positive  
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43 509 RT-PCR test. We use antibody positive status as an alternative to RT-PCR positive  
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45 510 status as a standard for assessing SARS-CoV-2 antibody assays and show strong  
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47 511 performance for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-  
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49 512 CoV-2 antibodies. User experience in future studies in the real world is important and  
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51 513 may alter the performance characteristics. Also, the effect of operator training will have  
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53 514 direct effects upon test performance. We welcome further clinical evaluation of the  
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55 515 AbC-19 LFIA in large cohorts of symptomatic and asymptomatic individuals alongside  
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3 516 large studies assessing vaccination outcomes in individuals to fully validate its  
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5 517 implementation across all intended use cases.  
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9  
10 519 **Declarations**

11  
12 520 **Ethics approval and consent to participate**

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14 521 All study participants provided informed consent. This study was approved by Ulster  
15  
16 522 University Institutional Ethics committee (REC/20/0043), South Birmingham REC (The  
17  
18 523 PANDEMIC Study IRAS Project ID: 286041Ref 20/WM/0184) and adhered to the  
19  
20 524 Declaration of Helsinki and Good Clinical Practice.  
21  
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24 525 **Patient and Public Involvement**

25  
26 526 Patients or the public were not involved in the design, or conduct, or reporting, or  
27  
28 527 dissemination plans of our research.  
29  
30

31 528 **Consent for publication**

32  
33 529 Not applicable.  
34

35 530 **Dissemination to participants and related patient and public communities.**

36  
37 531 Links to this work will be included on the study website  
38  
39 532 (<https://www.ulster.ac.uk/coronavirus/research/research-output/pandemic-study>) and  
40  
41 533 participants will be alerted that the work has been published.  
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44

45 534 **Data sharing**

46  
47 535 Data are available on reasonable request to the corresponding author.  
48

49 536 **Competing interests:**

50  
51 537 At the time of this study TM and JML acted as advisors to CIGA HealthCare, an  
52  
53 538 industrial partner in the UK Rapid Test Consortium. No personal financial reward or  
54  
55 539 remuneration was received for this advisory role. At the time of submission of this  
56  
57 540 manuscript TM and JML no longer held these advisory positions.  
58  
59  
60

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3 541 All other authors have no potential conflict of interest to report.  
4

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6

7  
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9  
10 544 practice (UU-UK-RTC-2020-001). The authors have not been paid or financially  
11  
12 545 benefitted from this study.  
13

14 546 The advisory roles within CIGA Healthcare were unpaid temporary roles. This  
15  
16 547 manuscript and associated data within this paper has only been used to build  
17  
18 548 confidence into the overall device design and performance assessment of the UK RTC  
19  
20 549 AbC-19 devices and such work was never commissioned for any government  
21  
22 550 contractual consideration.  
23

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26 551 **Authors' contributions:**  
27

28 552 TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR,  
29  
30 553 SM and KYN analysed data, KB performed all statistical analyses/interpretations and  
31  
32 554 produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided  
33  
34 555 SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and provided  
35  
36 556 Blood Transfusion cohort samples. TM, RP and AN coordinated participant  
37  
38 557 recruitment, consent and sampling. WB and JML developed online consent forms,  
39  
40 558 questionnaires and databases. LR, JM, AK, AA, GEW, DH, SS, CCS performed  
41  
42 559 sample collection and processing. LR and TM wrote the manuscript, with significant  
43  
44 560 contributions from JM, AN and KB. All authors reviewed and approved the final  
45  
46 561 manuscript.  
47  
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51 562

52  
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56  
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58  
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3 566 draws whilst ensuring the highest possible level of safety to the participants. We are  
4  
5 567 also grateful to Kingsbridge Private Hospital Group for sponsorship and providing  
6  
7  
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9  
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11  
12 570 space and equipment during the pandemic within a locked down University.  
13  
14  
15 571

## 572 **References**

- 16  
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698 **Table 1: Antibody level ratios for assays over time**

699 Antibody level ratios for assays over time show varying peak levels depending on test.

700 Calculated by first establishing the median per time period, then calculating log<sub>2</sub> ratio

701 for each period versus each respective assay positivity cut-off.

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

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

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

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3 706 **Figure Legends**  
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8 708 **Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott**

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10 709 **and c) Roche immunoassays.** Pearson  $\chi^2$  test was used to assess correlations. The  
11  
12 710 results for each test were log transformed to ensure results follow a normal distribution.

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14 711 Negative agreement shown as blue dots, red dots show positive agreement for the

15  
16 712 two immunoassays, whilst black dots show disagreement and grey dots as the

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18 713 EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4.

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20 714 n=880. The graphs show positive correlations between all immunoassays evaluated,

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22 715 with the fewest disagreement of results between the Log of Roche and the Log of

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24 716 EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.  
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30 718 **Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c)**

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32 719 **Abbott, relative to weeks since first reported symptoms or positive PCR result**

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34 720 **(where data available, n=685).** RT-PCR positive individuals are denoted by red dots,

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36 721 while individuals with time since symptom data are denoted in black. Dashed lines

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38 722 delineate  $\log_e$  equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott

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40 723 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result

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42 724 between the two lines). Black bars indicate median, within IQR (interquartile range)

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44 725 boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on

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46 726  $1.5 \times$  IQR (interquartile range).  
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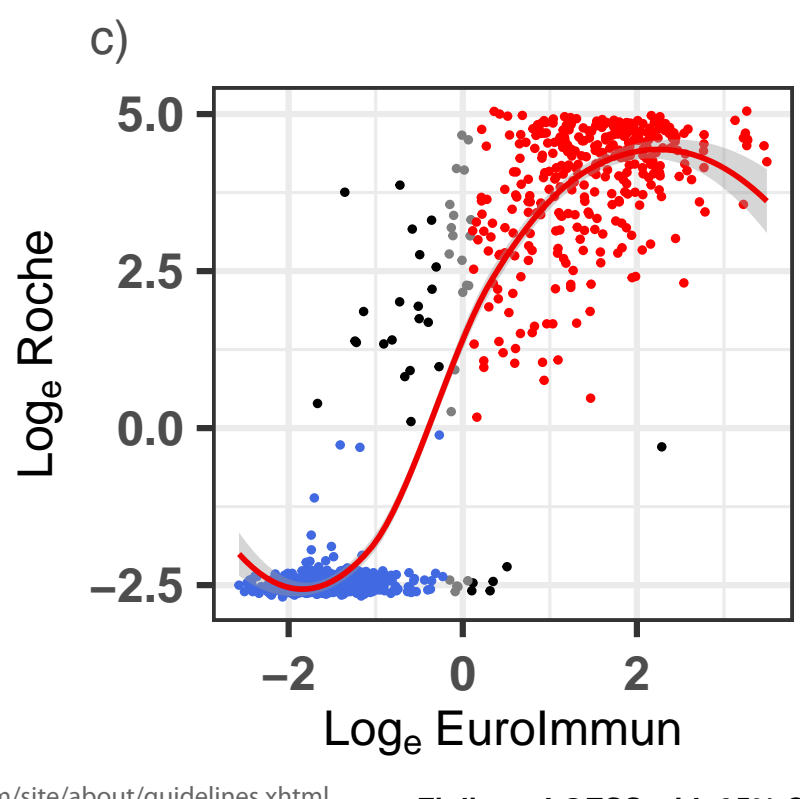
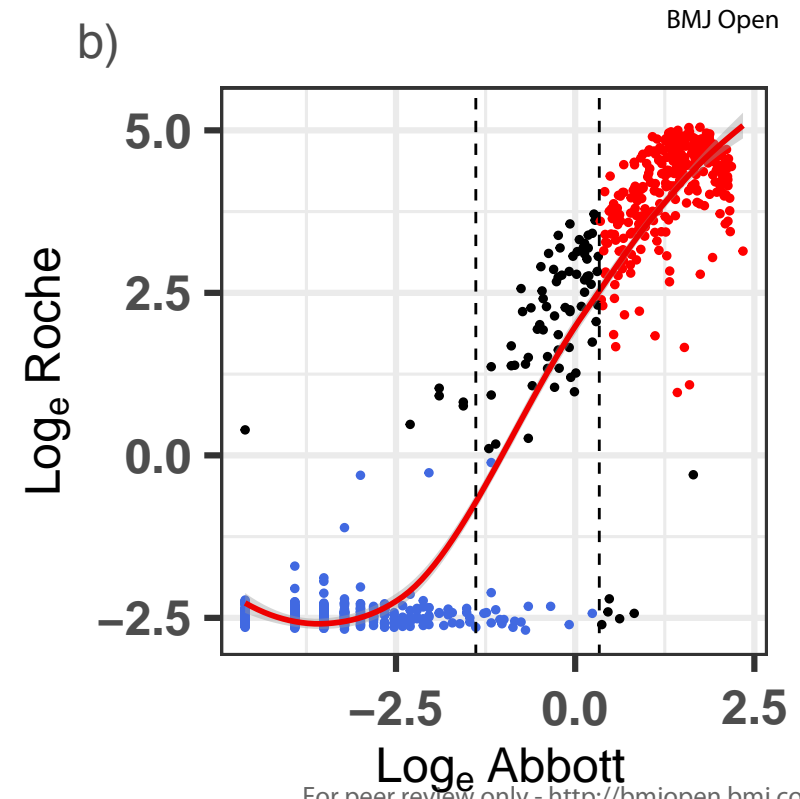
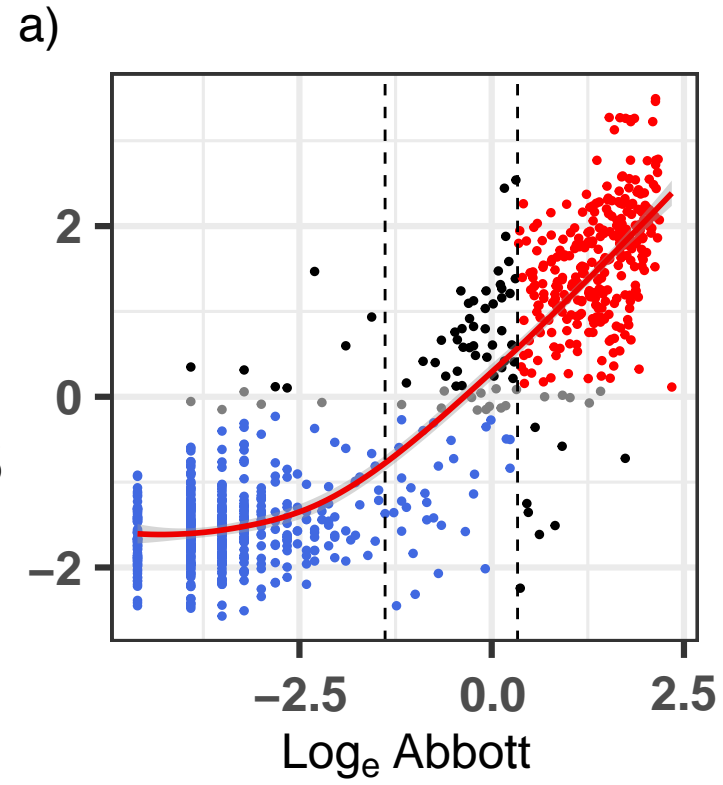
49  
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52 728 **Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche**

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54 729 **and c) Abbott scores.** Box plots overlaid on scatter plot, comparing AbC-19 TT3 test

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56 730 scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line  
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3 731 of best fit with 95% confidence interval shaded in grey. Black bars indicate median,  
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5 732 within IQR (interquartile range) boxes for EuroImmuno/Roche/Abbott value. Red  
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8 733 triangles indicate outliers, based on  $1.5 \times$  IQR (interquartile range).  
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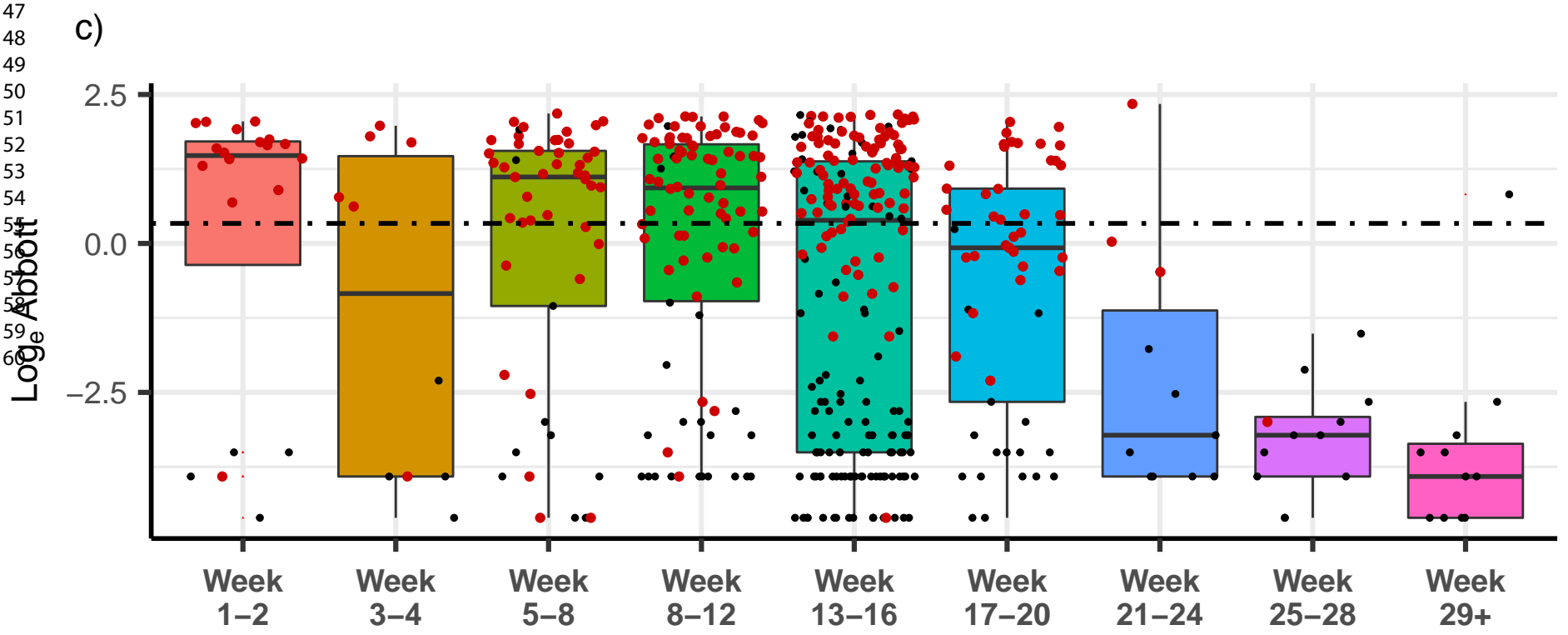
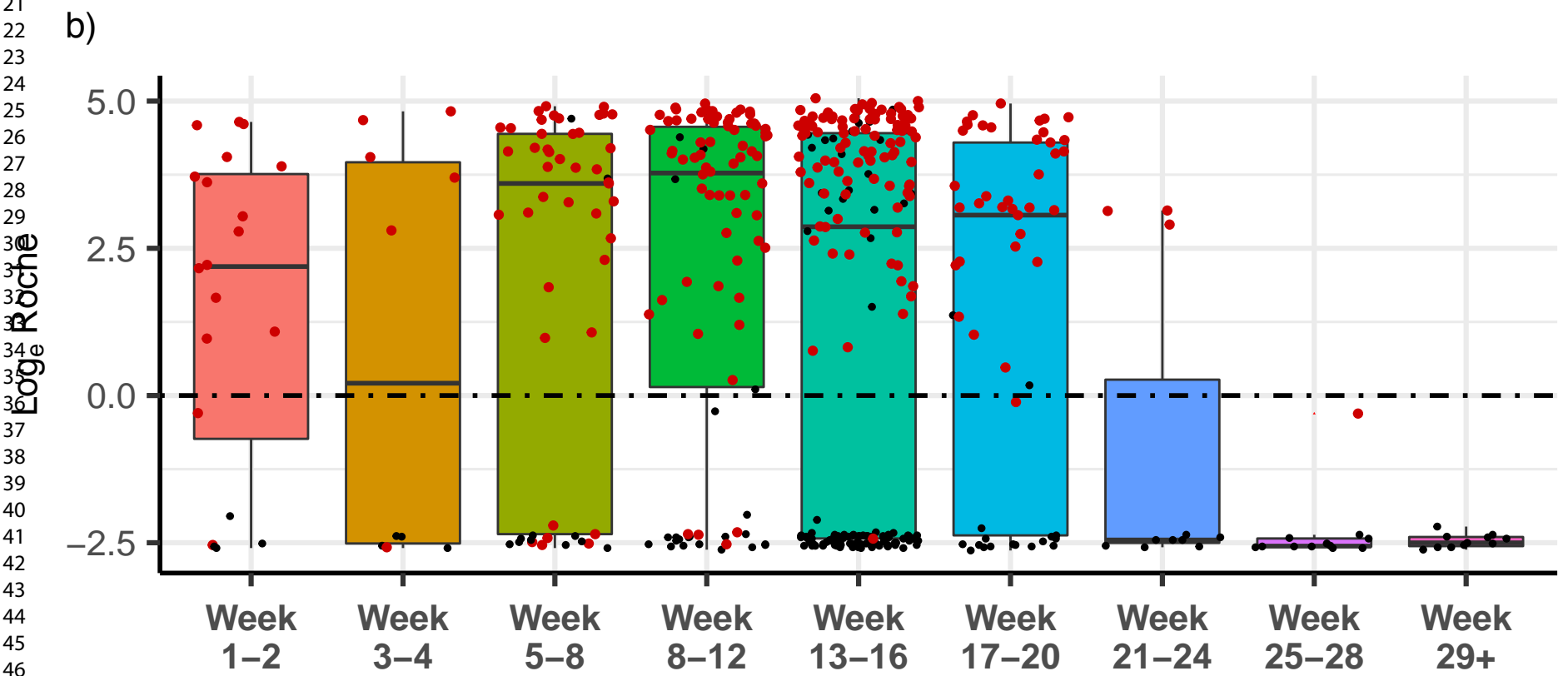
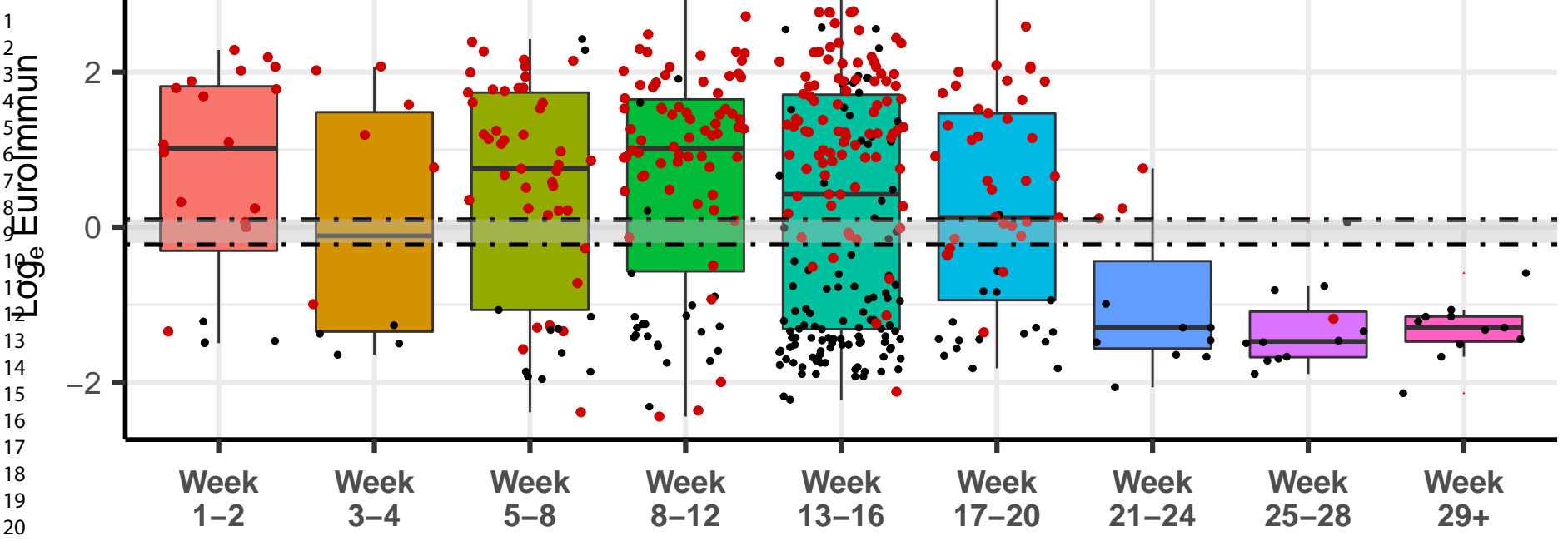
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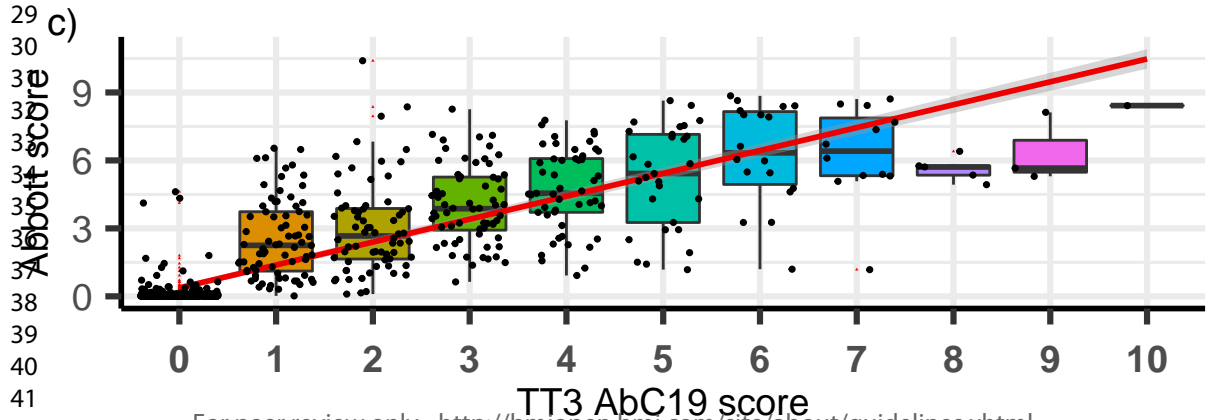
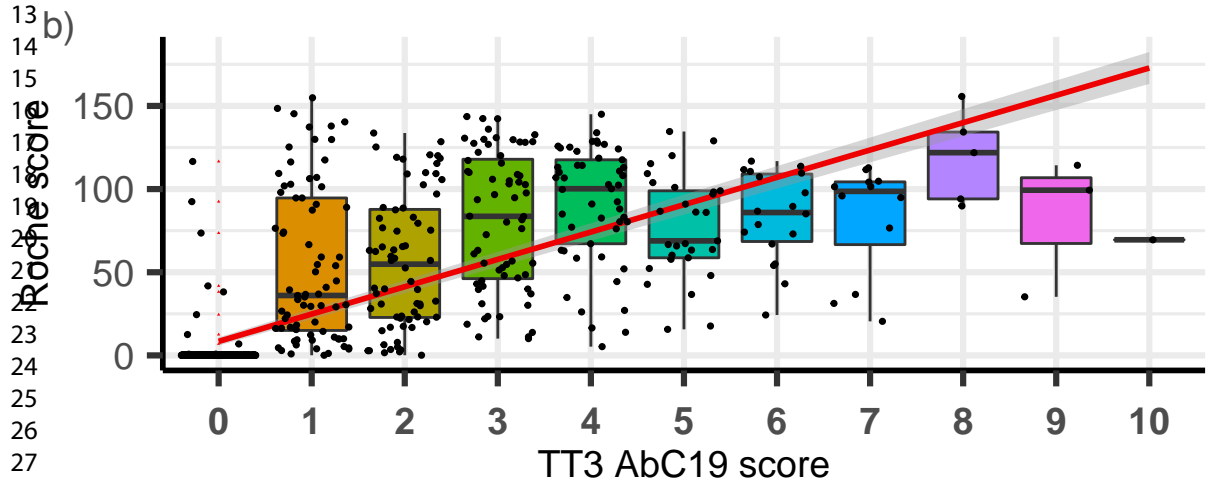
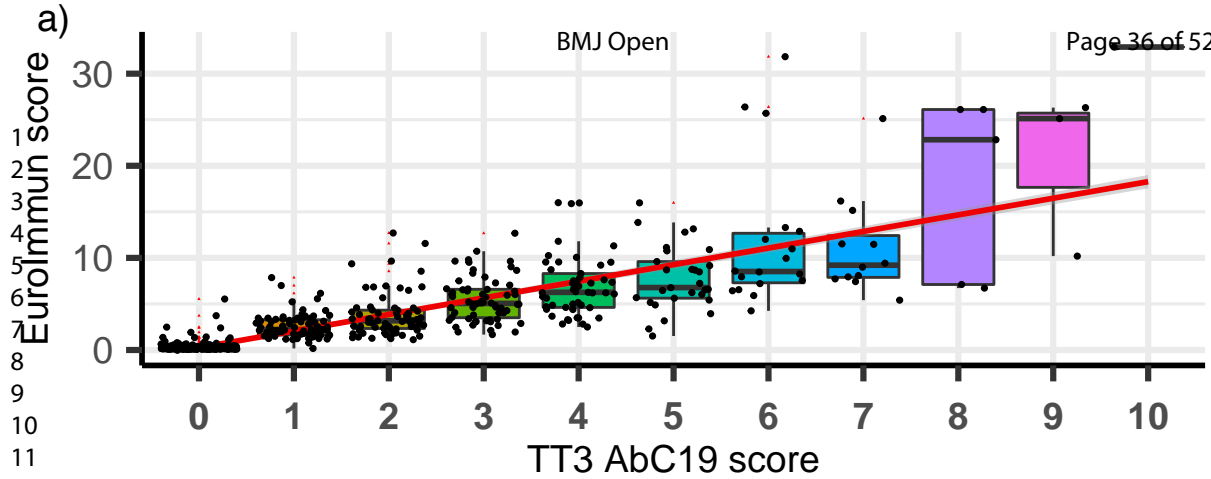


- Category**
- Both negative
  - Both positive
  - Disagreement
  - EuroImmun borderline

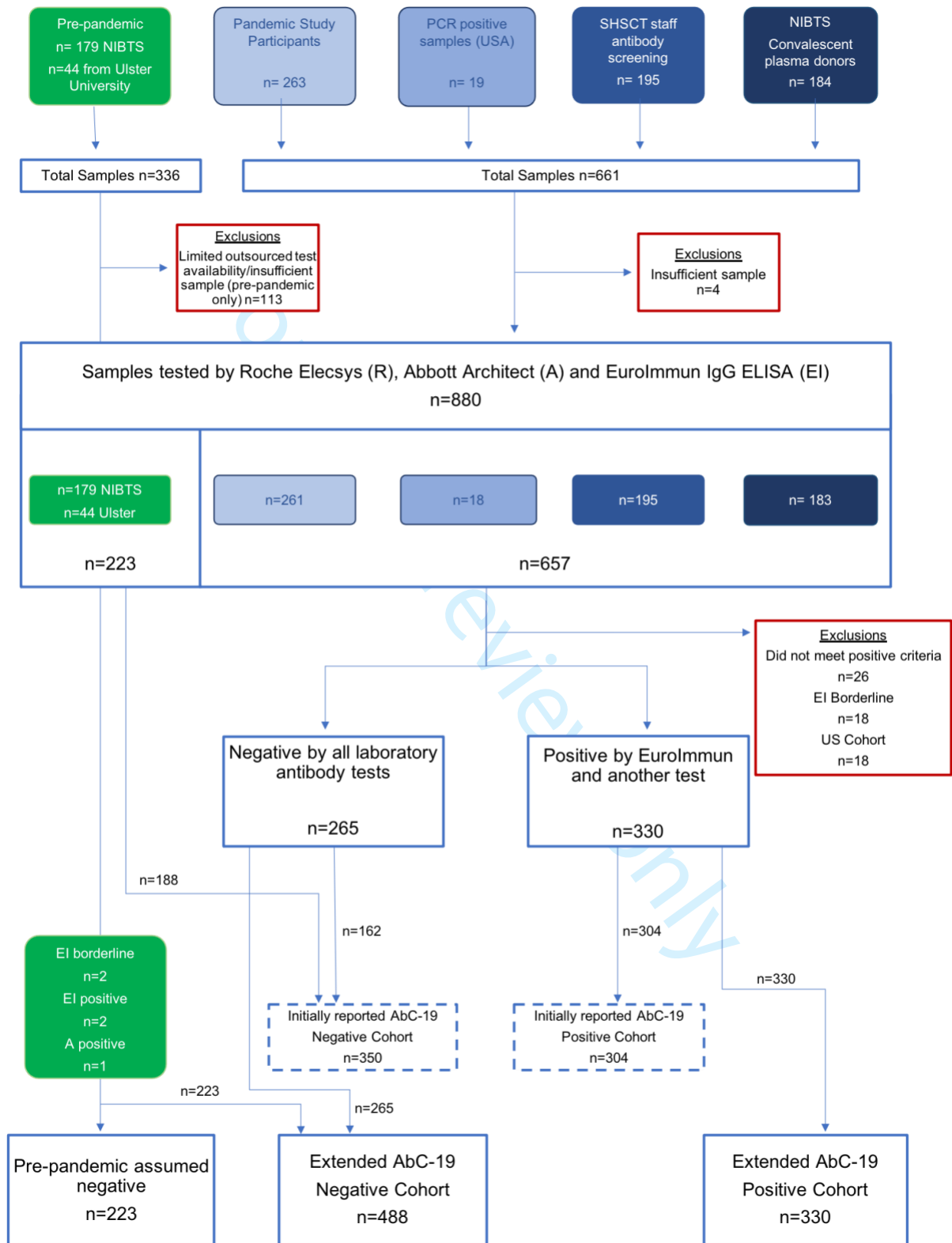
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Fit lines, LOESS with 95% CI  
Vertical lines mark Abbott test range 0.25–1.4





Supplementary Materials



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3 **Figure S1: Flow of participant plasma samples through cross-sectional study.**

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5 All available samples from participants within each cohort, and the included and  
6 excluded samples at all stages. Freeze thaw cycles were closely monitored for all  
7  
8 excluded samples at all stages. Freeze thaw cycles were closely monitored for all  
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10 sample aliquots. Pre-pandemic samples taken forward for Roche, Abbott and  
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12 EuroImmun testing were selected based on aliquot volume and availability.  
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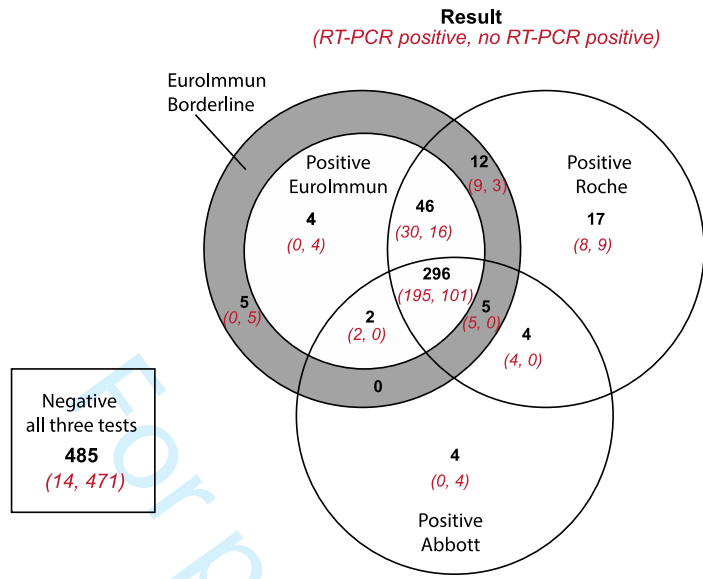


44 **Figure S2: Visual Score card for quantitative interpretation of AbC-19 LFIA test**  
45 **bands.** A scale of 0 (not pictured, negative-no test line visible) to 10 (positive-  
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47 strongest test line). Any LFIA scoring 1 or above was classified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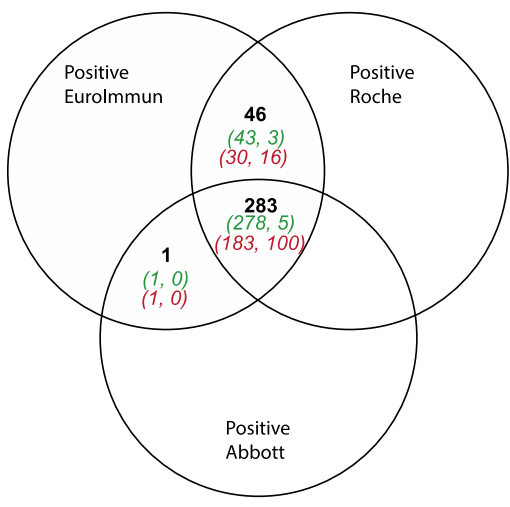
a)

Laboratory immunoassays (n=880)



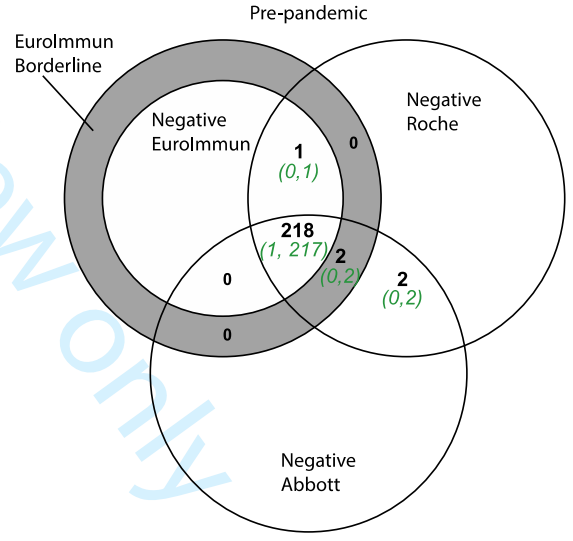
b) Positive cohort (n=330)

**Result**  
*(AbC-19 positive, AbC-19 negative)*  
*(RT-PCR positive, no RT-PCR positive)*

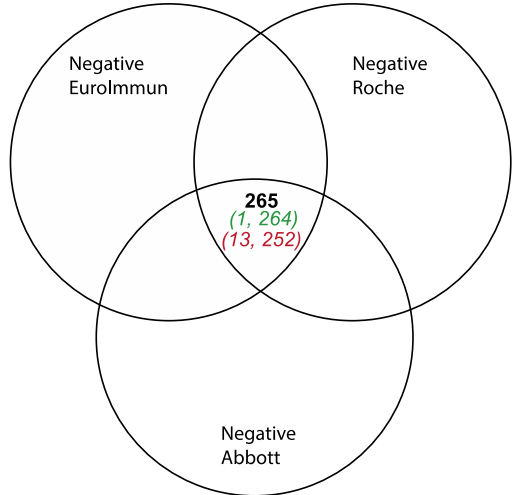


c) Negative cohort (n=488)

**Result**  
*(AbC-19 positive, AbC-19 negative)*  
*(RT-PCR positive, no RT-PCR positive)*

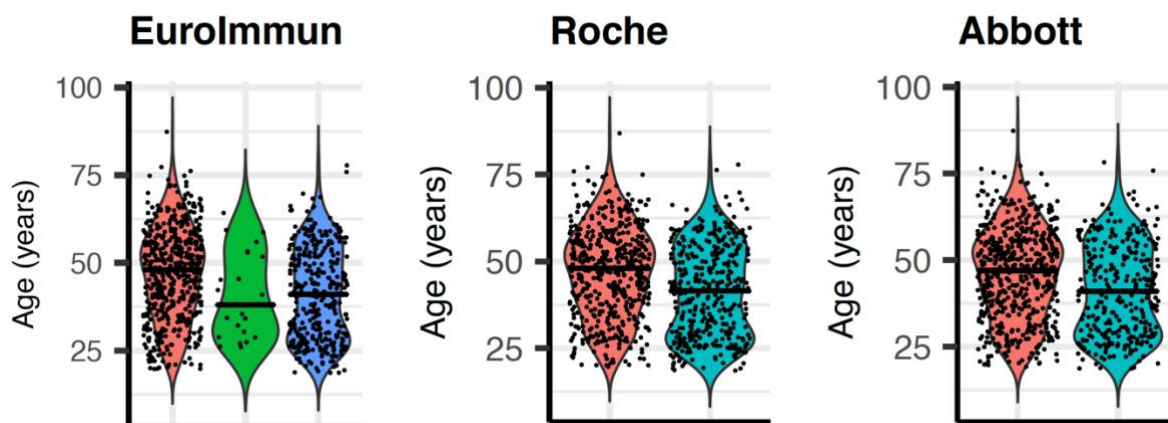


Collected 2020



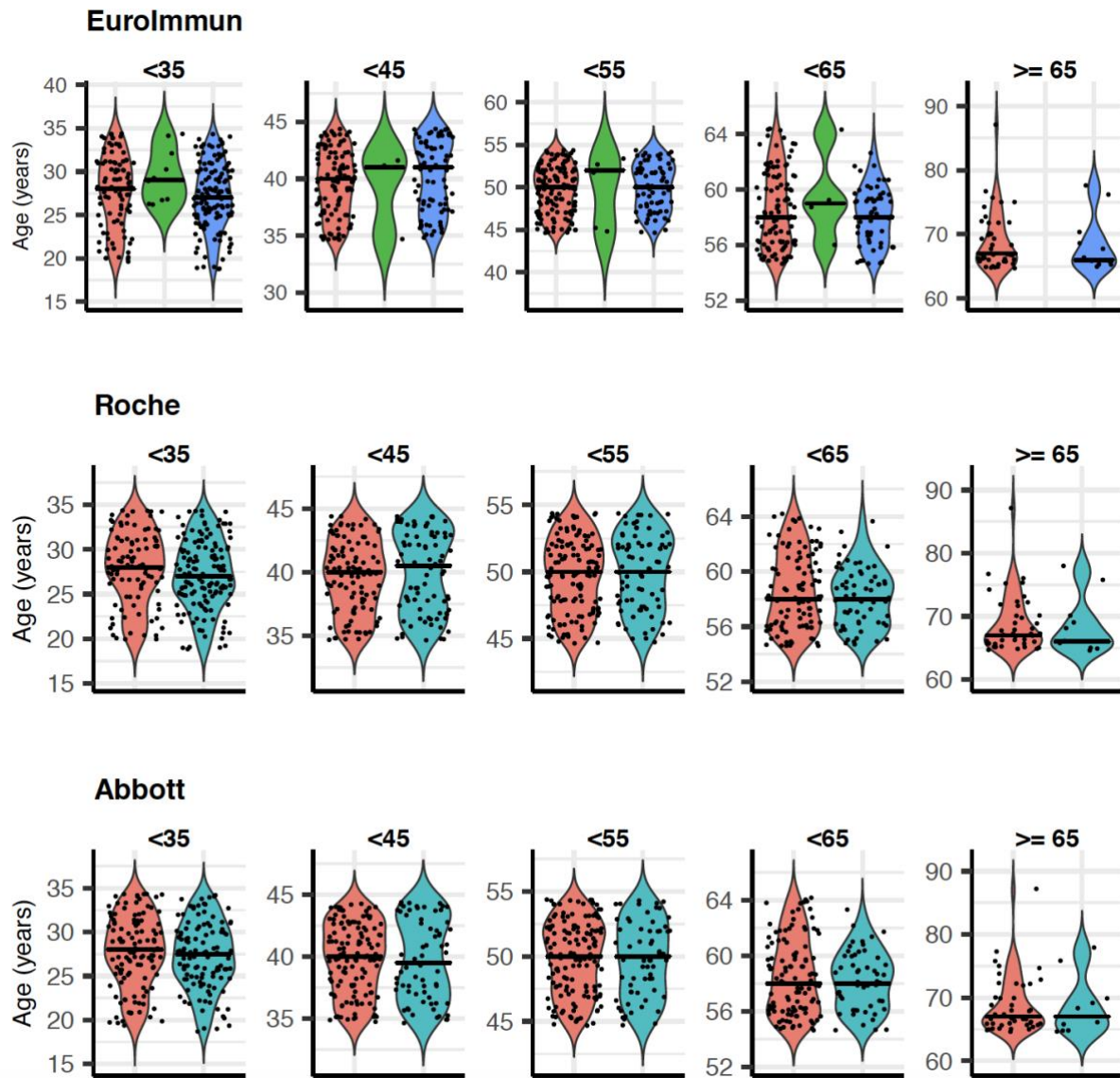
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3 **Figure S3: Venn diagrams demonstrating result overlap between laboratory**  
4 **assays in a) the initial immunoassay cohort (n=880), b) the positive and c)**  
5 **negative cohorts assessed with AbC-19 TT3.** Result in each circle overlap in bold,  
6 (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below. Where AbC-  
7  
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10 (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below. Where AbC-  
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13 19 was analysed, (AbC-19 positive, AbC-19 negative) denoted in green.  
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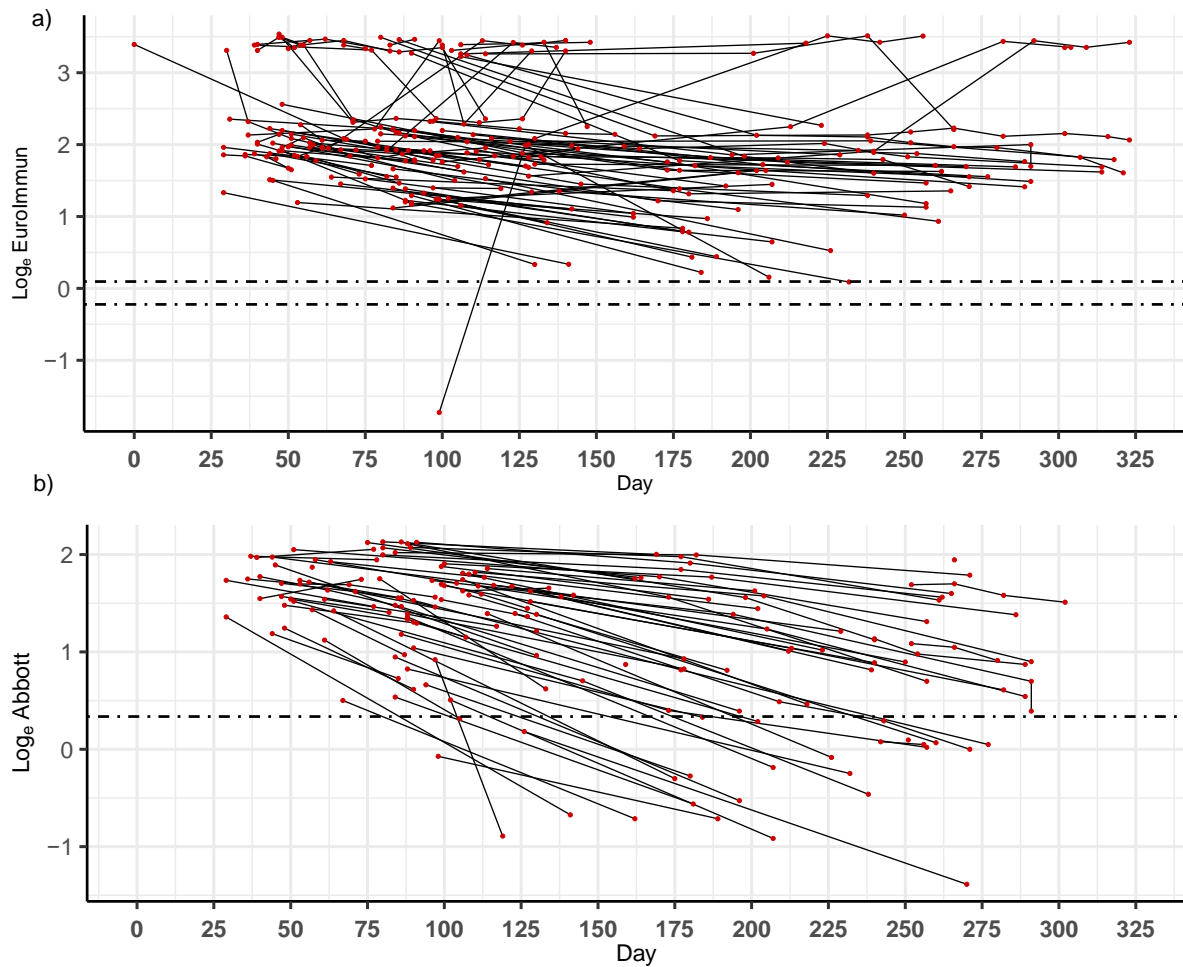
**Figure S4: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.**

The above graphs allow comparison of the distributions and probability density of ages for EuroImmune, 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.

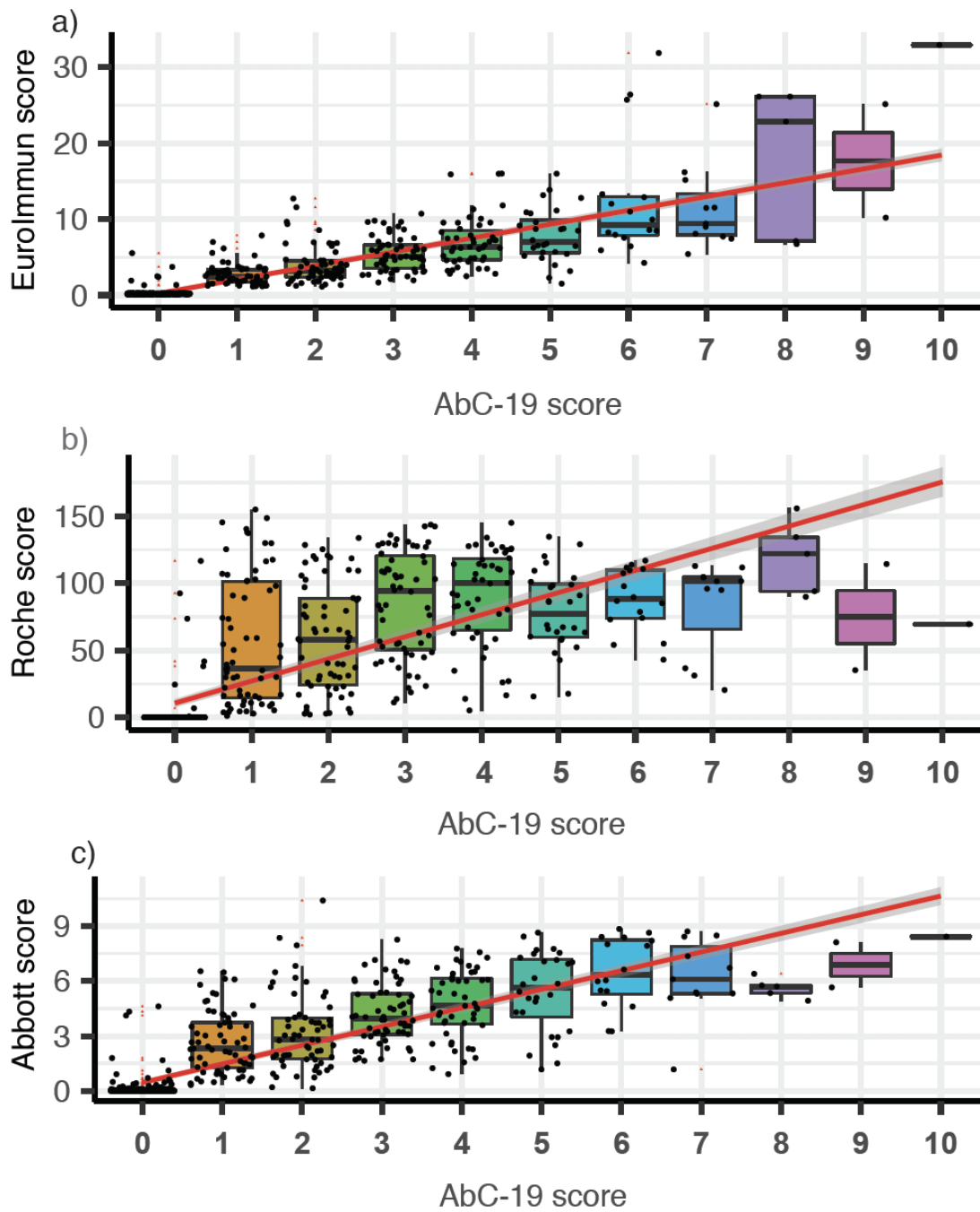


**Figure S5: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.**

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  $\geq 65$  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).

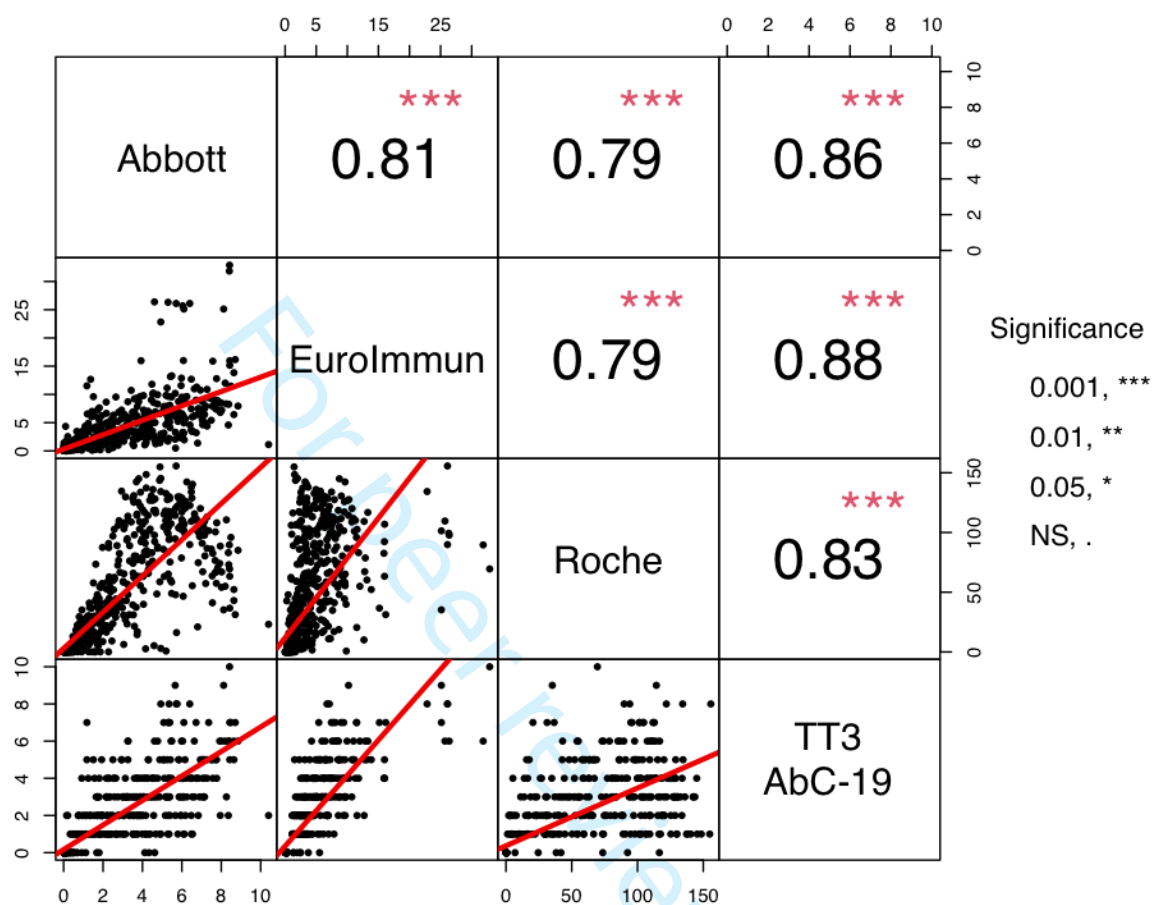


**Figure S6: Longitudinal analysis of convalescent plasma donor sequential samples (2-9 samples per individual) by a) EuroImmunit ELISA or b) Abbott immunoassay.** a)  $n=101$  individuals, grey shading indicates borderline region, upper dotted line indicates positivity threshold (1.1), lower dotted 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.



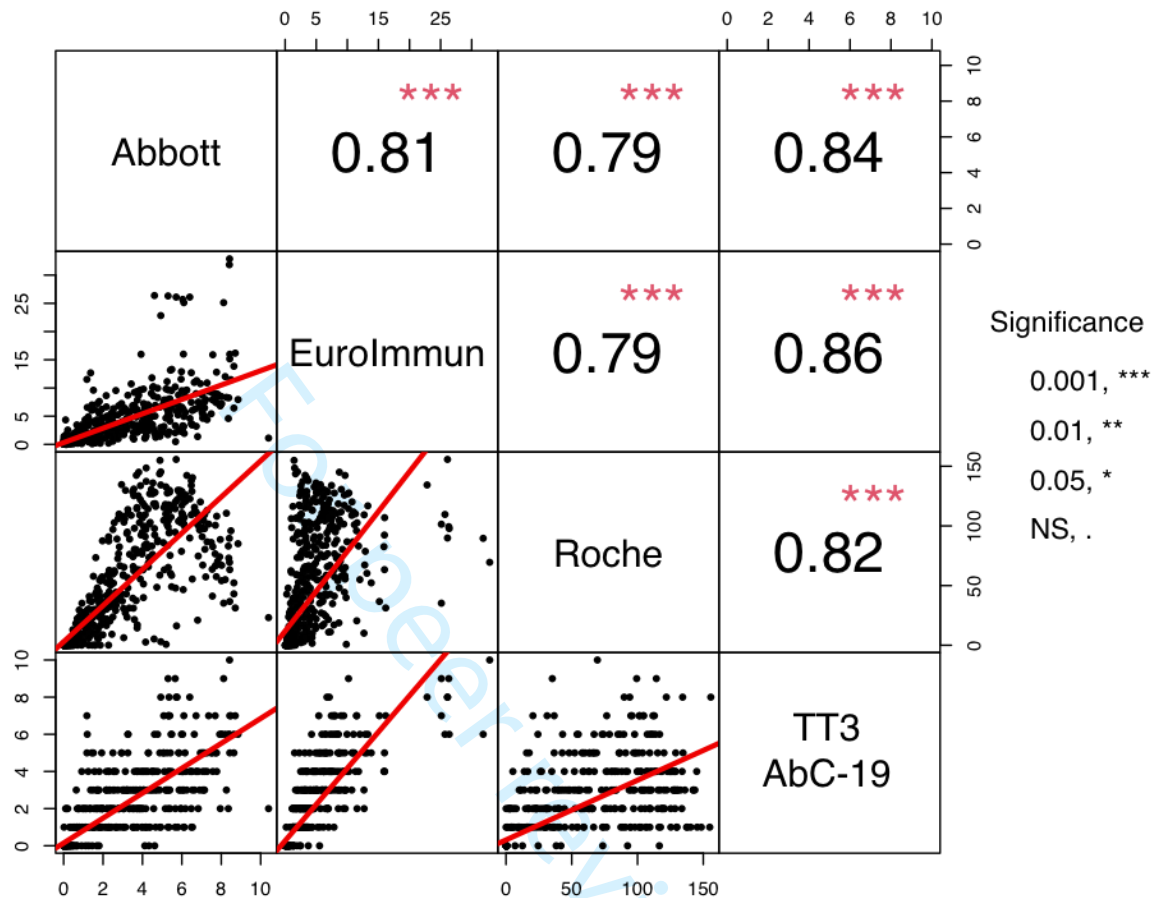
Linear fit line with 95% CI

**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  $1.5 \times$  IQR (interquartile range).



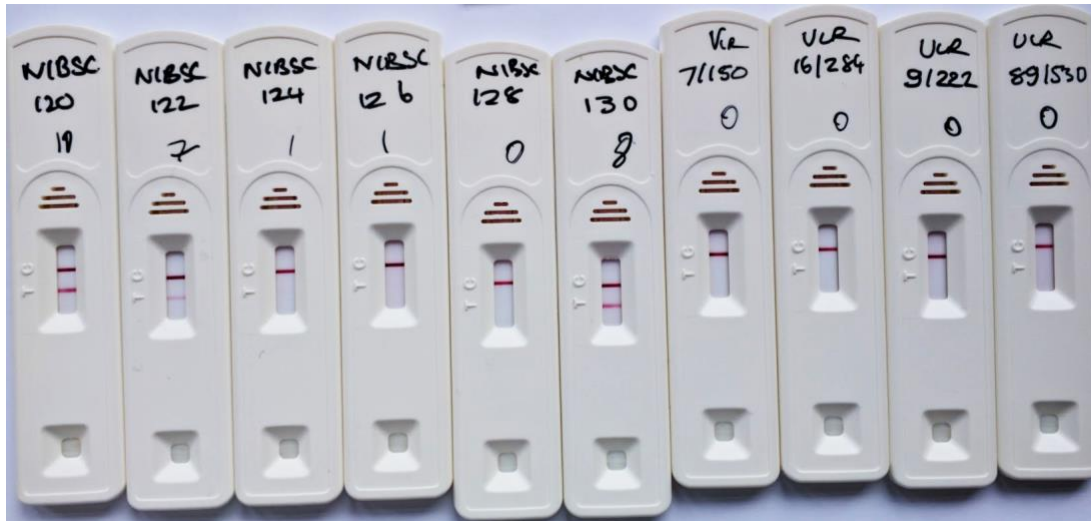
**Figure S8: Correlation matrix between Abbott, EuroImmun, Roche and initially reported AbC-19 cohort (n=654) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at  $p < 0.05$ . All immunoassays were 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 LFAs 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
<b>EuroImmun ELISA</b>	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
<b>Roche Elecsys immunoassay</b>	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
<b>Abbott Architect SARS-CoV-2</b>	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
<b>3 AbC-19</b>	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%)
Eurolmmun	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.

EuroImmune Anti-SARS-CoV-2 ELISA-IgG (EuroImmune, 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 EuroImmune calibrator, with a score of < 0.8 determined negative,  $\geq 0.8$  to <1.1 borderline and  $\geq 1.1$  positive. For samples provided by NIBTS, EuroImmune 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  $\geq 1.0$  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

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3 20/B764-01). Results are reported by dividing the sample result by the calibrator result.  
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5 The result unit for the SARS-CoV-2 IgG assay is Index (Sample/Calibrator). A ratio of  
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7 < 1.4 is determined negative and  $\geq 1.4$  is determined positive.  
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### 10 11 12 *Analytical specificity and sensitivity assessment* 13

14 Four virology samples (H5N1 influenza serology 7/150, RSV serology 16/284,  
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16 Influenza B 9/222 and Bordetella Pertusis 89/530) were obtained from NIBSC  
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18 (National Institute for Biological Standards, Herts, UK). An additional 30 serology  
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20 samples from known virus infections were a kind gift from Sugentech, Seoul, Korea.  
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22 15 of these virology samples were obtained from Trina (Trina Bioreactives AG,  
23  
24 Switzerland) from 5 different individuals per virus (Influenza A IgG, Influenza B IgG  
25  
26 and RSV IgG). A further 15 of these virology samples were obtained from AbBaltris,  
27  
28 Kent from 5 different individuals per virus (Haemophilus Influenza IgG, Seasonal  
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30 Coronavirus NL63 and 229E Seasonal Coronavirus). All these serology samples  
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32 alongside a panel of 6 external standard research reagents (Table S4; NIBSC; Cat:  
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34 20/118 and 20/130) were assessed on the TT3 AbC-19 LFIA to confirm analytical  
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36 specificity and sensitivity.  
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Section & Topic	No	Item	Reported on page #
<b>TITLE OR ABSTRACT</b>			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
<b>ABSTRACT</b>			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
<b>INTRODUCTION</b>			
	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
<b>METHODS</b>			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
<i>Participants</i>	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
<i>Test methods</i>	9	Whether participants formed a consecutive, random or convenience series	6
	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
<i>Analysis</i>	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
<b>RESULTS</b>			
<i>Participants</i>	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
<i>Test results</i>	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
<b>DISCUSSION</b>			
	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
<b>OTHER INFORMATION</b>			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents

For peer review only



# BMJ Open

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

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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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Professor James McLaughlin [jad.mclaughlin@ulster.ac.uk](mailto:jad.mclaughlin@ulster.ac.uk)

## 36 **Abstract**

### 37 *Objective*

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

### 42 *Design*

43 Nationwide serological study.

### 44 *Setting*

45 Northern Ireland, UK, May 2020- February 2021.

### 46 *Participants*

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

### 52 *Main Outcome Measures*

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

### 58 *Results*

59 We detected persistence of SARS-CoV-2 IgG antibodies for up to 10 months post  
60 infection, across a minimum of two laboratory immunoassays. On the known positive  
61 cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58%  
62 (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-  
63 99.95%).

### 64 *Conclusions*

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

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3 69 19 rapid test for SARS-CoV-2 spike protein IgG antibody detection in a laboratory-  
4 based setting.  
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9 72 **Strengths and Limitations**

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11 73 • Strength - This paper describes a non-clinical laboratory evaluation and  
12 comparison of the ability of three different immunoassays to detect SARS-CoV-  
13 74 2 antibodies in the same samples, detecting different subtypes of antibodies  
14 against different targets of the viral antigenic repertoire, that does not rely on  
15 75 PCR-positivity as definition of expected test outcome, to provide a panel of  
16 known antibody positive and antibody negative serology for evaluation of newly  
17 76 developed immunoassays.  
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29 81 • Strength - This study demonstrates AbC-19 lateral flow point of care detection  
30 of IgG antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the  
31 antibodies made in response to the vaccines used globally, in a large cohort of  
32 82 subjects, more than 10 months post infection, across a broad age range (18-  
33 78 years).  
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41 86  
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43 87 • Strength - This study assesses correlation between approved laboratory-based  
44 assays and the AbC-19 lateral flow point of care lateral flow test for the  
45 88 detection of SARS-CoV-2 antibodies in characterised cohorts of known positive  
46 and negative plasma samples in an evaluation conducted according to MHRA  
47 89 guidelines during a pandemic.  
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3 94 • Strength- Longitudinal data detecting IgG antibodies more than 10 months from  
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5 95 infection was collected as sequential samples over time through a convalescent  
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7 96 plasma donation program.  
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10 97  
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12 98 • Limitation- This study was conducted in a standardised setting with very  
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14 99 experienced users on plasma characterised as positive or negative for the  
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16 100 presence of antibodies using a reference standard, alongside one other assay  
17  
18 101 which may introduce a possible spectrum bias and may not reflect the true  
19  
20 102 performance metrics of the assay evaluated when translated to real life  
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22 103 settings, using finger prick blood samples and in which pre-test probability  
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24 104 would impact greatly on positive and negative predictive values.  
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### 106 **Keywords**

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

### 109 **Introduction**

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

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

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3 119 infection, the number of SARS-CoV-2 genes analysed, and the proportion of  
4  
5 120 asymptomatic individuals tested (6,7). Lockdown measures and “flattening the curve”  
6  
7 121 strategies in the UK meant many infected individuals were instructed to self-isolate  
8  
9 122 and were not offered a diagnostic RT-qPCR, with much of the testing limited to patients  
10  
11 123 admitted to hospital, who perhaps reflect a more severely infected cohort.  
12  
13 124 Consequently, a potentially large number of cases were unconfirmed or undetected  
14  
15 125 (8).

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18  
19 126 The ability to accurately detect SARS-CoV-2 specific antibodies, which develop after  
20  
21 127 an immune response is evoked, is vital for building biobanks of convalescent sera for  
22  
23 128 treatment, monitoring immune response to infection alongside surveillance studies  
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25 129 and assessing responses to vaccination programmes.

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29 130 Commercial serology immunoassays are mostly laboratory-based and measure IgG  
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31 131 antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIA),  
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33 132 require a finger prick blood sample and can be used at point-of-care (POC) or in the  
34  
35 133 home; particularly important in the context of lockdown enforcement during the  
36  
37 134 pandemic. A limited number of laboratory-based chemiluminescence immunoassays  
38  
39 135 are approved for use in the UK including the Roche Elecsys Anti-SARS-CoV-2  
40  
41 136 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region (Roche  
42  
43 137 Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay against the  
44  
45 138 same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

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49 139 The complexities of the humoral immune response to SARS-CoV-2 is a much-  
50  
51 140 debated topic. In a US study, approximately one in 16 individuals lacked detectable  
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53 141 IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR  
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55 142 confirmed infection (9). Patients who remain asymptomatic may mount a humoral  
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57 143 immune response which is short-lived, with detectable levels of antibody falling



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3 144 rapidly (10). This, alongside the lack of RT-PCR test availability across the UK has  
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5 145 hindered development of well characterised gold standard serology test for IgG  
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7 146 antibodies to SARS-CoV-2.  
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9  
10 147 Herein, we describe the use of Roche and Abbott commercial immunoassays, as well  
11  
12 148 as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike  
13  
14 149 (antigenic) protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-  
15  
16 150 pandemic and pandemic COVID-19 blood samples (n=880) from within Northern  
17  
18 151 Ireland and report on longevity of IgG antibodies detected. Furthermore, we follow IgG  
19  
20 152 antibody levels in convalescent plasma donors (n=101 individuals) for up to 11  
21  
22 153 months. Currently, there is no gold standard assay for comparison, therefore we aimed  
23  
24 154 to establish a reference based on a positive COVID-19 antibody status. We present  
25  
26 155 results of a laboratory evaluation of the UK-RTC AbC-19 with a target condition of  
27  
28 156 antibodies against a cohort of 330 known IgG antibody positive samples according to  
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30 157 this 'positive by two' system and 488 negative samples (223 pre-pandemic assumed  
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32 158 negative and 265 known negative) for IgG to SARS-CoV-2.  
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## 160 **Methods**

### 162 **Participant samples**

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

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3 169 The first 800 respondents who expressed interest were provided with an online patient  
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5 170 information sheet, consent form and health questionnaire and invited to register to  
6  
7 171 attend a clinic. Participants were eligible for the study if they were over 18 years of  
8  
9 172 age. Exclusion criteria included anyone with a blood disorder or contraindication to  
10  
11 173 giving a blood sample, or anyone currently exhibiting symptoms of COVID-19. To  
12  
13 174 enrich the cohort for samples potentially positive for SARS-CoV-2 IgG antibody,  
14  
15 175 further participants were invited if they had previously tested PCR positive or had the  
16  
17 176 distinctive symptom of loss of taste and smell. Blood sampling clinics were held at  
18  
19 177 locations around Northern Ireland between May and July 2020 resulting in collection  
20  
21 178 of 263 10ml EDTA plasma samples from 263 separate study participants. Additional  
22  
23 179 anonymised plasma samples were obtained from Southern Health and Social Care  
24  
25 180 Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood Transfusion  
26  
27 181 Service (NIBTS, n=184) through convalescent plasma programs. NIBTS convalescent  
28  
29 182 plasma samples continued to be collected throughout 2020-early 2021, with a total of  
30  
31 183 n=897 from n=676 individuals, including n=183 samples from the cross-sectional  
32  
33 184 cohort. Individuals from this program with a positive RT-PCR result and EuroImmun  
34  
35 185 starting value >6 were sequentially sampled over a period of up to 46 weeks resulting  
36  
37 186 in a cohort of n=101 individuals, n=296 samples (including n=47 individuals from the  
38  
39 187 cross-sectional cohort).  
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49 189 Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster  
50  
51 190 University ethics committee approved studies with ongoing consent and from NIBTS  
52  
53 191 (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-  
54  
55 192 thaw cycles for all analyses reported within this manuscript.  
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## 194 **Clinical information**

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

201

## 202 **Laboratory-based immunoassays**

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

205

## 206 **UK-RTC AbC-19 LFIA**

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

215

## 216 **Statistical analysis**

217 As per Daniel (11) a minimum sample size based on prevalence can be calculated

218 using the following formula:  $n = \frac{Z^2 P(1 - P)}{d^2}$ , where n = sample size, Z = Z statistic for a

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3 219 chosen level of confidence,  $P$  = estimated prevalence, and  $d$  = precision. Assuming  
4  
5 220 a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the  
6  
7 221 required sample size at 99% confidence ( $Z = 2.58$ ) to be 240 individuals. If the true  
8  
9 222 prevalence is lower, 5%, the estimated required sample size given a precision of  
10  
11 223 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200  
12  
13 224 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody  
14  
15 225 immunoassays(12).

16  
17 226 Statistical analysis was conducted in in R v 4.0.2(13). To assess discordance between  
18  
19 227 test results, data was first filtered to include individuals with an Abbott test result in the  
20  
21 228 range  $\geq 0.25$  &  $\leq 1.4$ , with a 2 x 2 contingency table produced that comprised all  
22  
23 229 possible combinations of [concordant|discordant] test results [within|outside of] this  
24  
25 230 range. A p-value was derived via a Pearson  $\chi^2$  test after 2000 p-value simulations via  
26  
27 231 the stats package.

28  
29 232 AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc  
30  
31 233 Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To  
32  
33 234 compare test result (Positive|Negative) to age, a binary logistic regression model was  
34  
35 235 produced with test result as outcome – a p-value was then derived via  $\chi^2$  ANOVA. To  
36  
37 236 compare time against test result (encoded continuously), a linear regression was  
38  
39 237 performed. We calculated median per time-period and then converted these to log  
40  
41 238 [base 2] ratios against the positivity cut-off for each assay. All plots were generated  
42  
43 239 via ggplot2 or custom functions using base R(14).

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## 46 47 241 **Results**

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49 242 We analysed samples from a mixed cohort of individuals from the general public  
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51 243 (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations  
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3 244 and research studies (n=223) and through a convalescent plasma program (n=183).  
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5 245 Antibody levels in plasma from these 880 individuals were assessed using the three  
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7 246 SARS-CoV-2 immunoassays; EuroImmun IgG, Roche Elecsys IgG/IgM/IgA and  
8  
9 247 Abbott Architect IgG (Table S1, Figure S3). This included a cohort of 223 pre-  
10  
11 248 pandemic plasma samples collected and stored during 2017 to end of May 2019 to  
12  
13 249 determine assay specificity. Of the 657 participants whose samples were collected  
14  
15 250 during the pandemic, 267 (40.64%) previously tested RT-PCR positive with a range of  
16  
17 251 7-173 days since diagnosis. A total of 225 participants gave time since self-reported  
18  
19 252 COVID-19 symptoms, with a range of 5-233 days from symptom onset, whilst 195 had  
20  
21 253 no symptom or PCR data available. Samples collected in 2020 (n=657) ranged from  
22  
23 254 19-78 years of age with a median (IQR) of 43 years ( $\pm 22$ ), and n=454 were female  
24  
25 255 and n=200 male (n=3, not disclosed). Pre-pandemic samples (n=223) ranged from 20-  
26  
27 256 87 years of age with median (IQR) of 50 years ( $\pm 20$ ) and consisted of n=88 female  
28  
29 257 and n=135 male.

### 258 *Laboratory based antibody immunoassays*

259 A positive result for antibody on one or more of the three laboratory immunoassays  
30  
31 260 was recorded for 385/657 (58.6%) participants who provided a sample during the  
32  
33 261 pandemic. By EuroImmun ELISA, 346 were positive, 20 borderline and 291 were  
34  
35 262 negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott  
36  
37 263 determined 310 positive and 347 negative (Table S2, Figure S3). The median age  
38  
39 264 across all age groups combined was lower for participants testing positive across each  
40  
41 265 of the immunoassays (median [sd] for positive versus negative, respectively:  
42  
43 266 EuroImmun, 41 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41  
44  
45 267 [13.18] vs 47 [13.09]). (Figure S4,  $p < 0.0001$ ). When segregated by age group,  
46  
47 268 however, differences were less apparent in certain groups (Figure S5). Excluding the

1  
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3 269 pre-pandemic cohort, this gap reduced but remained statistically significant  
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5 270 EuroImm, 41 [13.18] vs 45 [12.49]; Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41  
6  
7 [13.26] vs 44 [12.63]) ( $p < 0.01$ ) (median [sd] for positive versus negative). Of note, out  
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9 of 267 individuals with a previous positive RT-PCR result for SARS-CoV-2 viral RNA,  
10  
11 272 14 (5.2%, Figure S3a) did not show detectable antibodies by any of the three  
12  
13 273 immunoassays, with no association found with age, gender or time between test and  
14  
15 274 blood draw (data not shown).  
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18  
19 276 The three commercial laboratory immunoassays provide a ratio value that increases  
20  
21 277 with IgG antibody titre. When correlation between these values is assessed, good  
22  
23 278 overall agreement is observed between the three immunoassays (Figure 1, Figure  
24  
25 279 S5). As highlighted by Rosadas *et al.*, we also see significant disagreement in the  
26  
27 280 Abbott 0.25-1.4 range when compared to EuroImm and Roche (Figure 1a,b; chi-  
28  
29 281 square p-values: EuroImm vs Abbott,  $p < 0.001$ ; Roche vs Abbott,  $p < 0.001$ )(15).  
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### 35 36 37 283 *Duration of humoral response to SARS-CoV-2*

38  
39 284 In a cross-sectional analysis of antibodies over time, we found IgG antibodies could  
40  
41 285 still be detected in individuals (excluding pre-pandemic) across all three  
42  
43 286 immunoassays used up to week 20 (day 140) (Figure 2). We note a statistically  
44  
45 287 significant decrease in signal with respect to time across each assay (p-value  
46  
47 288 [estimate slope]): EuroImm,  $p = 0.028$ [-0.823]; Roche,  $p = 0.002$  [-0.125]; Abbott,  
48  
49 289  $p < 0.0001$  [-3.673]. These remained statistically significant after adjustment for age.  
50  
51 290 Antibody levels (expressed as a ratio of median result per timepoint divided by  
52  
53 291 positivity cut off; Table 1) peaked at Week 1-2 for EuroImm (1.33) and Abbott  
54  
55 292 (1.64), though reached highest levels at Week 8-12 when measured by Roche  
56  
57 293 (5.45). By week 21-24, median score for all tests had dropped below the positivity  
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3 294 cut off, though a small number of samples remained above the positive cut off at  
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5 295 these later timepoints (Figure 2).  
6  
7 296 Samples from the NIBTS convalescent plasma program continued to be collected  
8  
9 297 throughout 2020-early 2021. A total of n= 897 samples from n=676 individuals were  
10  
11 298 collected, 744/883 tested by EuroImmun were positive (>1.1, with values range of  
12  
13 299 0.051-34.361), 556/749 tested by Abbott were positive (>1.4, with values ranging  
14  
15 300 from 0.01-8.85). Individuals with a positive RT-PCR result and a EuroImmun result  
16  
17 301 >6 were sequentially sampled (with median 3, range 2-9 samples per individual) and  
18  
19 302 analysed by both EuroImmun (n=101 individuals), and Abbott immunoassays (n=75  
20  
21 303 individuals). Median age (IQR) for this cohort is 51 years ( $\pm 21$ ) with a range from 18-  
22  
23 304 70 years and n=27 female, n=74 male. Longitudinal analysis shows persistence of  
24  
25 305 detectable IgG antibodies until up to 302 days (43 weeks) by Abbott immunoassay  
26  
27 306 (at which point this assay was discontinued at NIBTS) and 323 days (46 weeks) by  
28  
29 307 EuroImmun ELISA, with a gradual decline over time (Figure S6). None of the  
30  
31 308 individuals who were initially positive by Euroimmun SARS-CoV-2 S1 IgG assay  
32  
33 309 dropped to below the EuroImmun positivity threshold (>1.1) over the course of the  
34  
35 310 follow-up while 26 who were initially positive by Abbott SARS-CoV-2 NP IgG fell  
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37 311 below the Abbott threshold (>1.4).  
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### 313 *UK-RTC AbC-19*

314 Using the commercial immunoassays described we established a well characterised  
315 serology sample set of 'known positive' and 'known negative' for IgG antibodies to  
316 SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.  
317 AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore  
318 required all samples to be positive by the EuroImmun SARS-CoV-2 IgG ELISA, which



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2  
3 319 likewise detects antibodies against the S1 domain (16). To develop this characterised  
4  
5 320 cohort, samples were also required to be positive by a second immunoassay (Roche  
6  
7 321 or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG  
8  
9 322 antibody, we assessed 350 plasma samples from participants classed as 'known  
10  
11 323 negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from  
12  
13 324 individuals confirmed to be negative across all three laboratory assays (Roche,  
14  
15 325 EuroImmun, Abbott). Using these positive n=304 and negative n=350 antibody  
16  
17 326 cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of  
18  
19 327 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the  
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21 328 AbC-19 LFIA (Table 2).

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26 329 Given a recent report of lower specificity in the AbC-19 LFIA (17) and the possibility  
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28 330 of introducing sample bias, we revised our inclusion criteria for the negative cohort.  
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30 331 For the pre-pandemic cohort, we included samples from all 223 individuals,  
31  
32 332 regardless of results on other laboratory immunoassays. When this assumed  
33  
34 333 negative pre-pandemic cohort was used for laboratory evaluation for target condition  
35  
36 334 of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 2). We  
37  
38 335 expanded the negative cohort to include all samples that matched our criteria  
39  
40 336 (samples collected during the pandemic to be negative by all three laboratory assays  
41  
42 337 and all pre-pandemic samples regardless of other immunoassay results). The  
43  
44 338 specificity observed on this extended negative cohort of 488 samples was 99.59%  
45  
46 339 (98.53% to 99.95%, Table 2). For sensitivity analysis on a positive cohort (samples  
47  
48 340 positive by EuroImmun and one other test), we were able to analyse all samples  
49  
50 341 previously untested due to limited testing capacity and tested a positive cohort of 330  
51  
52 342 samples giving a sensitivity of 97.58% (95.28% to 98.95%, Table 2). When we  
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54 343 sorted samples analysed in both negative (n=488) and positive cohorts (n=330) by  
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3 344 RT-PCR status and assessed AbC-19 LFIA sensitivity by including only those that  
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5 345 were RT-PCR positive (n=227), the test showed a sensitivity of 92.07% (87.76%-  
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7 346 95.23%, Table S3). However, of the n=18 RT-PCR positive individuals negative for  
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9 347 IgG antibodies by AbC-19, n=12 showed no detectable antibodies by all three  
10  
11 348 laboratory assays (EuroImmun, Roche or Abbott), suggesting that antibodies are not  
12  
13 349 present in those samples.  
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17 350  
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19 351 When used as intended by the public, the AbC-19 LFIA provides binary  
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21 352 positive/negative results. However, when assessing LFIA in the laboratory, each test  
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23 353 line was scored against a scorecard by three independent researchers (0 negative, 1-  
24  
25 354 10 positive; Figure S2). When compared to quantitative outputs from the Abbott,  
26  
27 355 EuroImmun and Roche assays, the AbC-19 LFIA shows good correlation (Abbott  
28  
29 356  $r=0.84$  [ $p<0.001$ ]; EuroImmun  $r=0.86$  [ $p<0.001$ ]; Roche  $r=0.82$  [ $p<0.001$ ]; Figure 3,  
30  
31 357 Figure S7-Figure S9).  
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### 359 *Analytical specificity and sensitivity of AbC-19 LFIA*

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38 360 We observed no cross-reactivity across samples with known H5N1 influenza,  
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40 361 Respiratory syncytial virus, Influenza A, Influenza B, Bordetella Pertussis,  
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42 362 Haemophilus Influenzae, Seasonal coronavirus NL63 and 229E on the AbC-19 LFIA  
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44 363 (n=34 samples, n=8 distinct respiratory viruses; Table S4). Against a panel of external  
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46 364 reference SARS-CoV-2 serology samples, the AbC-19 LFIA detected antibodies with  
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48 365 scores commensurate to the EuroImmun ELISA scores (Figure S10, Table S5).  
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## 56 367 **Discussion**

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3 368 Serological antibody immunoassays are an important tool in helping combat the  
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5 369 SARS-CoV-2 pandemic. The duration of the humoral immune response is of  
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8 370 particular importance, to inform an individual's protection following both natural  
9  
10 371 infection and vaccination. Using a large cohort of individuals across a wide age  
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12 372 range (18-78), we assessed antibody levels across up to three laboratory  
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14 373 immunoassays perform a cross-sectional and longitudinal analysis over time. Our  
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16 374 results show strong correlation between all three immunoassays, with shortcomings  
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18 375 in the Abbott system output 0.25-1.4 range, as described previously, suggesting an  
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20 376 overestimated positive cut-off (Figure 1) (15).  
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25  
26 378 Longitudinal studies on SARS-CoV-1 convalescent patients suggests that detectable  
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28 379 IgG can still be present as long as 2 years after infection (18). There are conflicting  
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30 380 reports of the longevity of the humoral response to SARS-CoV-2 infection which  
31  
32 381 differ in the make-up of the cohort studied, the assays used, and the length of time  
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34 382 since symptom onset. The longevity of IgG antibodies to both spike and  
35  
36 383 nucleocapsid protein more than 10 months after RT PCR positive status (and  
37  
38 384 beyond in a small number of samples, Figure 2, Figure S6) is consistent with that  
39  
40 385 observed in other recent studies(19–21). In this study, samples were collected  
41  
42 386 through a convalescent plasma program (Figure S6), with individuals selected for  
43  
44 387 sequential plasma donation based on an initial high EuroImmune assay score. In  
45  
46 388 contrast to the time series analysis of healthcare workers recruited prospectively by  
47  
48 389 Manisty *et al.*, we observed no cases where Euroimmune ELISA-measured anti-Spike  
49  
50 390 antibody levels fell below threshold, whilst a large number of Abbott measured anti-  
51  
52 391 Nucleocapsid antibody levels dropped below the positivity threshold (34.7% 26/75).  
53  
54 392 However, this may be an overestimate given the shortcomings of the Abbott assay  
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3 393 described above (Figure 1) (22). In a similar longitudinal study of 51 symptomatic  
4  
5 394 participants, Dan *et al.* estimated that half-life ( $t_{1/2}$ ) for IgG-Spike (103 days) was  
6  
7 395 longer than that for IgG-Nucleocapsid (68 days), although with a considerable  
8  
9 396 overlap of 95% confidence intervals (23).

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11  
12 397 In our more diverse cross-sectional cohort, we also note a statistically significant  
13  
14 398 decline over time but levels remain detectable at 140 days (Figure 2). We note that  
15  
16 399 IgG levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as  
17  
18 400 Week 8-12 from first symptoms or a viral RNA RT-PCR positive result, though this  
19  
20 401 may be an artefact of lower number of participants at earlier timepoints (Table 1).  
21  
22 402 Robust antibody responses are produced in our cohorts across a wide age range  
23  
24 403 (18-78 years old, Figure 2, Figure S6). We detect a slightly but significantly lower  
25  
26 404 median age of participants testing positive (Figure S4); however, this is likely be due  
27  
28 405 to cohort characteristics and not a true reflection of the population or indication of  
29  
30 406 test performance.  
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38 408 A difficulty faced in validation of antibody diagnostic assays has been access to  
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40 409 samples with known SARS-CoV-2 antibody status. As previously described, there is  
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42 410 no clear gold standard reference against which to assess SARS-CoV-2  
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44 411 immunoassays. A positive RT-PCR test has been used previously to indicate previous  
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46 412 (COVID-19) SARS-CoV-2 infection, though this approach is limited by a high rate of  
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48 413 false negatives and positives in RT-PCR testing, failure in some cases to develop IgG  
49  
50 414 antibodies (sero-silence or lack of antibody against the same antigenic component of  
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52 415 the virus as the immunoassay uses as a capture antigen) and the lack of RT-PCR  
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54 416 testing availability early in the pandemic (3,5,24). SARS-CoV-2 IgG antibodies were  
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56 417 undetectable in 14 of 267 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA  
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3 418 positive participants in this study. It is unclear if this is due to insufficient/absent  
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5 419 antibody production in these individuals at the time the sample was taken, or due to a  
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8 420 false positive PCR result which may occur in the UK at a rate between 0.8- 4.0% (6).  
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10 421 Self-assessment of symptoms for COVID-19 (disease) is a poor indicator of previous  
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12 422 infection, even amongst healthcare workers (25). Additionally, the kinetics of a SARS-  
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14 423 CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus  
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17 424 with time, contributing to false negative RT-PCR test results for individuals who may  
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19 425 be late to present for virus detection tests (5,26).  
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24 427 To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-  
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26 428 CoV-2 antibody in a laboratory evaluation, we developed a reference standard for  
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28 429 SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar  
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30 430 approach was used in a recent seroprevalence study in Iceland, whereby two positive  
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32 431 antibody results were required to determine a participant sample as positive for SARS-  
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34 432 CoV-2 antibody (24). Our evaluation of performance metrics for the UK-RTC AbC-19  
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36 433 LFIA to detect antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59%  
37  
38 434 specificity. In an evaluation of the AbC-19 tests, Mulchandani *et al.* observed a  
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40 435 specificity of 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report  
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42 436 a sensitivity of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a  
43  
44 437 previous RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys  
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46 438 antibody test, which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid  
47  
48 439 portion of SARS-CoV-2 (25). In RT-PCR positive individuals from our cohorts, the  
49  
50 440 AbC-19 test showed a similar sensitivity (92.07%, 87.76%- 95.23%, Table S3).  
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52 441 However, we demonstrate the drawbacks of this approach given that in 12 of 18 AbC-  
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54 442 19 false negatives, none of the four immunoassays used (EuroImmuno, Roche, Abbott  
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3 443 or AbC-19) detected antibodies, suggesting either a false RT-PCR result, a failure to  
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5 444 produce IgG antibodies or sero-reversion before sample collection in these individuals.  
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7 445 Another recent evaluation of the AbC-19 LFIA by Moshe *et al.* determined a sensitivity  
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9 446 of (100% (98.1-100%)) on laboratory sera, using a composite reference standard of  
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11 447 antibody positive by either Spike protein ELISA or hybrid DABA assay and specificity  
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13 448 of 99.8% (98.9-100%) against pre-pandemic samples. However, when AbC-19  
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15 449 performance was analysed on matched finger-prick and serum samples against the  
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17 450 same antibody standard, a lower sensitivity was observed (finger-prick 69% (53.8-  
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19 451 81.3%), serum 92% (80-97.7%)) (27).  
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26 453 In our study, strong correlation was observed in quantitative score between results on  
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28 454 all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA  
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30 455 (Figure S8, S9). This is to be expected, given both the AbC-19 LFIA and EuroImmun  
31  
32 456 ELISA detect IgG antibodies against spike protein. Importantly, for the assessment of  
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34 457 immunity to prior natural infection as well as to immunisation, IgG antibodies against  
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36 458 SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA and AbC-  
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38 459 19 LFIA are known to correlate with neutralizing antibodies, which may confer future  
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40 460 immunity (23,28,29). Previous evaluations of sensitivity and specificity reported by  
41  
42 461 Public Health England (PHE), showed a EuroImmun sensitivity of 72% and specificity  
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44 462 of 99%, Abbott with sensitivity of 92.7% and specificity of 100% and Roche with  
45  
46 463 sensitivity of 83.9% and specificity of 100% (30–32). The PHE analyses for each of  
47  
48 464 these tests used previous infection (RT-PCR positive status) as a reference standard,  
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50 465 the limitations of which are discussed above.  
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3 467 In the use of characterised 'known positive' and 'known negative' cohorts, one  
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5 468 limitation of this study is its potential for spectrum bias, whereby our positive-by-two  
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7 469 reference system may artificially raise the threshold for positive sample inclusion,  
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9 470 possibly resulting in the overestimation of the sensitivity of any test evaluated (33).  
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11 471 However, similar issues have been raised when using previous RT-PCR result or  
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13 472 definitive COVID-19 symptoms as inclusion criteria given these will likely skew a  
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15 473 cohort towards more severe disease, especially given issues of RT-PCR availability  
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17 474 outside of hospital settings during the first wave (5). Importantly, our mixed origin of  
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19 475 samples forming the cohort provides a positive cohort for assessing assay sensitivity  
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21 476 that includes individuals from the general public, healthcare workers and from  
22  
23 477 convalescent plasma programmes. In the absence of a clear gold standard test, our  
24  
25 478 system relies on no single test (each with their individual shortcomings) and instead  
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27 479 takes an average of three. Our analysis of specificity on only pre-pandemic individuals  
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29 480 (n=223) shows similar specificity (99.55%) to the larger mixed 'known negative cohort'  
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31 481 (n=488, sensitivity 99.59%). We also demonstrate a high level of analytical specificity  
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33 482 of the AbC-19 test with no cross-reactivity against a panel of other respiratory viruses,  
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35 483 including SARS-CoV-1 NL63 and 229E (Table S4).  
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484  
485 Our assessment of the AbC-19 LFIA in a laboratory setting, using characterised  
486 cohorts of known SARS-CoV-2 antibody positive and antibody negative plasma,  
487 shows good performance metrics for its ability to detect SARS-CoV-2 IgG antibodies  
488 following natural infection. We note our use of plasma from venous blood samples, as  
489 opposed to a finger prick blood sample as would be used in rapid testing scenarios  
490 (27). Additionally, when the AbC-19 LFIA was used on our cohort, a number of the  
491 positive results scored low (1/10 using the score card under laboratory conditions,

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3 492 Figure 3), with a faint test band visible to a trained laboratory scientist but perhaps  
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5 493 difficult to identify as positive by individuals performing a single test (Figure S10). This  
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8 494 faint line may be reflective of the longer time from infection for the Northern Ireland  
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10 495 cohort used. If the AbC-19 LFIA is to be used in clinical settings it is important to  
11  
12 496 determine if all users observe the same results as observed in this laboratory  
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14  
15 497 evaluation.

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17 498  
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19 499 This assessment of the AbC-19 LFIA does not provide data on how this test will  
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21 500 perform in a seroprevalence screening scenario, but instead provides metrics for the  
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23 501 performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as  
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25  
26 502 opposed to previous COVID-19 infection. An important potential use of the AbC-19  
27  
28 503 LFIA would be in monitoring the immune response to vaccination, with most vaccines  
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30 504 utilising SARS-CoV-2 Spike protein antigens (34).

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## 34 35 506 **Conclusion**

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37 507 We present a comprehensive analysis of pre-pandemic and two large pandemic  
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39 508 cohorts (more than 700 individuals) and in a longitudinal analysis showing that IgG  
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41 509 antibodies to SARS-CoV-2 antigens are detectable more than 10 months from positive  
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43 510 RT-PCR test. We use antibody positive status as an alternative to RT-PCR positive  
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45 511 status as a standard for assessing SARS-CoV-2 antibody assays and show strong  
46  
47 512 performance for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-  
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49 513 CoV-2 antibodies. User experience in future studies in the real world is important and  
50  
51 514 may alter the performance characteristics. Also, the effect of operator training will have  
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53 515 direct effects upon test performance. We welcome further clinical evaluation of the  
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55 516 AbC-19 LFIA in large cohorts of symptomatic and asymptomatic individuals alongside  
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3 517 large studies assessing vaccination outcomes in individuals to fully validate its  
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5 518 implementation across all intended use cases.  
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9  
10 520 **Declarations**

11  
12 521 **Ethics approval and consent to participate**

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14 522 All study participants provided informed consent. This study was approved by Ulster  
15  
16 523 University Institutional Ethics committee (REC/20/0043), South Birmingham REC (The  
17  
18 524 PANDEMIC Study IRAS Project ID: 286041Ref 20/WM/0184) and adhered to the  
19  
20 525 Declaration of Helsinki and Good Clinical Practice.  
21  
22

23  
24 526 **Patient and Public Involvement**

25  
26 527 Patients or the public were not involved in the design, or conduct, or reporting, or  
27  
28 528 dissemination plans of our research.  
29

30  
31 529 **Consent for publication**

32  
33 530 Not applicable.  
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35  
36 531 **Dissemination to participants and related patient and public communities.**

37  
38 532 Links to this work will be included on the study website  
39  
40 533 (<https://www.ulster.ac.uk/coronavirus/research/research-output/pandemic-study>) and  
41  
42 534 participants will be alerted that the work has been published.  
43

44  
45 535 **Data sharing**

46  
47 536 Data are available on reasonable request to the corresponding author.  
48

49  
50 537 **Competing interests:**

51  
52 538 At the time of this study TM and JML acted as advisors to CIGA HealthCare, an  
53  
54 539 industrial partner in the UK Rapid Test Consortium. No personal financial reward or  
55  
56 540 remuneration was received for this advisory role. At the time of submission of this  
57  
58 541 manuscript TM and JML no longer held these advisory positions.  
59  
60



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3 542 All other authors have no potential conflict of interest to report.  
4

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9  
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12 546 benefitted from this study.

13  
14 547 The advisory roles within CIGA Healthcare were unpaid temporary roles. This  
15  
16 548 manuscript and associated data within this paper has only been used to build  
17  
18 549 confidence into the overall device design and performance assessment of the UK RTC  
19  
20 550 AbC-19 devices and such work was never commissioned for any government  
21  
22 551 contractual consideration.  
23

24  
25  
26 552 **Authors' contributions:**

27  
28 553 TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR,  
29  
30 554 SM and KYN analysed data, KB performed all statistical analyses/interpretations and  
31  
32 555 produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided  
33  
34 556 SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and provided  
35  
36 557 Blood Transfusion cohort samples. TM, RP and AN coordinated participant  
37  
38 558 recruitment, consent and sampling. WB and JML developed online consent forms,  
39  
40 559 questionnaires and databases. LR, JM, AK, AA, GEW, DH, SS, CCS performed  
41  
42 560 sample collection and processing. LR and TM wrote the manuscript, with significant  
43  
44 561 contributions from JM, AN and KB. All authors reviewed and approved the final  
45  
46 562 manuscript.  
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58  
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2  
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13  
14  
15 572

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43 585 [1/fulltext#.X10EdBi-Ayk.mendeley](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30788-1/fulltext#.X10EdBi-Ayk.mendeley)  
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12 696 [f](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/890566/Evaluation_of_Abbott_SARS_CoV_2_IgG_PHE.pdf)  
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16 698 (IgG) serology assay for the detection of anti-SARS-CoV-2 antibodies  
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26 703 32. Public Health England. Evaluation of Roche Elecsys AntiSARS-CoV-2  
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3 714 **Table 1: Antibody level ratios for assays over time**

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5 715 Antibody level ratios for assays over time show varying peak levels depending on test.

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7 716 Calculated by first establishing the median per time period, then calculating log<sub>2</sub> ratio

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9 717 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+
<b>EuroImmuno</b>	-2.65	1.33	0.2	0.95	1.32	0.47	0.04	-2.01	-2.26	-2.01
<b>Roche</b>	-3.64	3.16	3.05	5.20	5.45	4.14	4.42	-3.54	-3.69	-3.61
<b>Abbott</b>	-5.54	1.64	-0.51	1.12	0.86	0.08	-0.59	-5.13	-5.13	-6.13
<b>Sample number (n=)</b>	223	20	10	52	90	202	53	11	12	11

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28 719 **Table 2: UK-RTC AbC-19 LFIA performance metrics against known antibody**

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31 720 **positive and known antibody negative cohorts.**

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

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3 722 **Figure Legends**  
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8 724 **Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott**  
9  
10 725 **and c) Roche immunoassays.** Pearson  $\chi^2$  test was used to assess correlations. The  
11  
12 726 results for each test were log transformed to ensure results follow a normal distribution.  
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14 727 Negative agreement shown as blue dots, red dots show positive agreement for the  
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16 728 two immunoassays, whilst black dots show disagreement and grey dots as the  
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18 729 EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4.  
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21 730 n=880. The graphs show positive correlations between all immunoassays evaluated,  
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23 731 with the fewest disagreement of results between the Log of Roche and the Log of  
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25 732 EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.  
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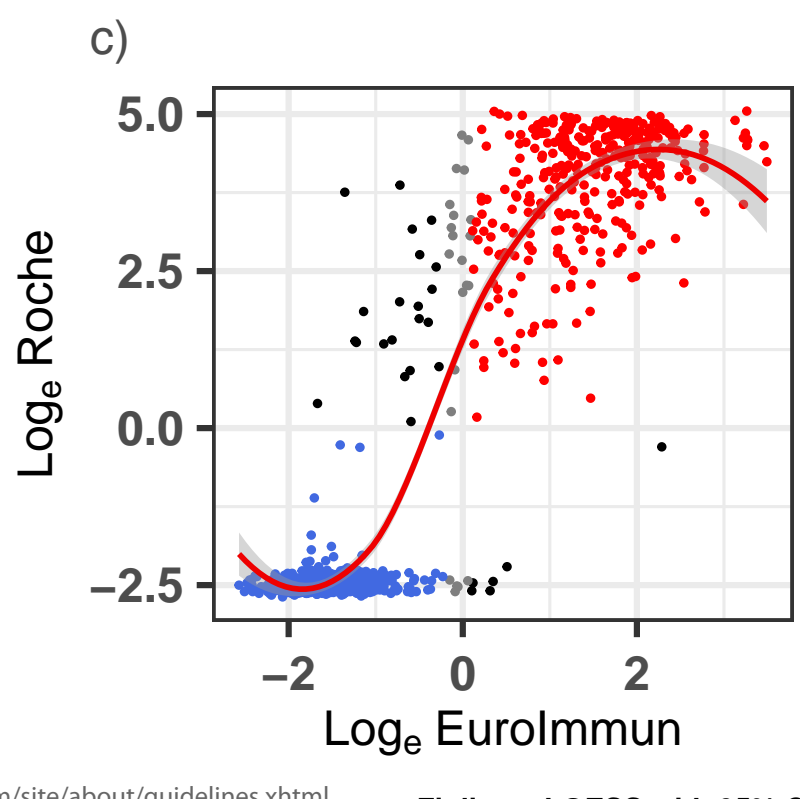
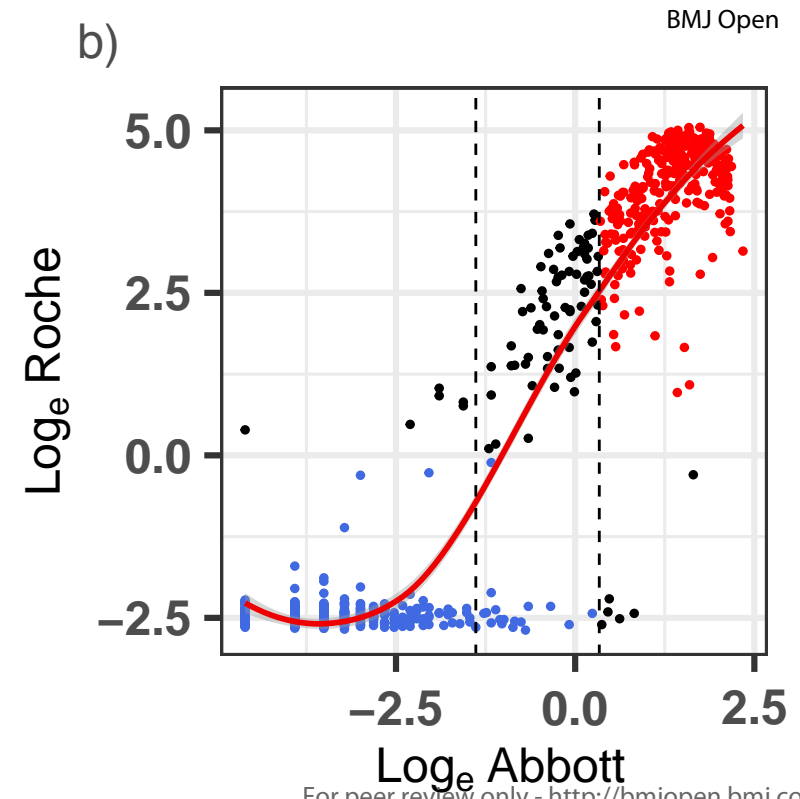
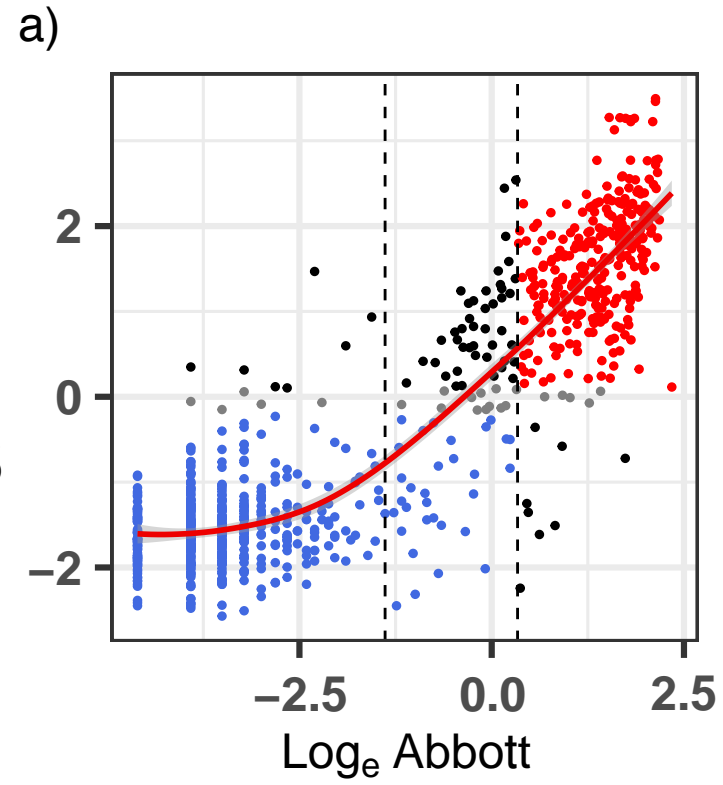
30 734 **Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c)**  
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32 735 **Abbott, relative to weeks since first reported symptoms or positive PCR result**  
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34 736 **(where data available, n=685).** RT-PCR positive individuals are denoted by red dots,  
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36 737 while individuals with time since symptom data are denoted in black. Dashed lines  
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38 738 delineate  $\log_e$  equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott  
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40 739 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result  
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42 740 between the two lines). Black bars indicate median, within IQR (interquartile range)  
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44 741 boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on  
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46 742  $1.5 \times$  IQR (interquartile range).  
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52 744 **Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche**  
53  
54 745 **and c) Abbott scores.** Box plots overlaid on scatter plot, comparing AbC-19 TT3 test  
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56 746 scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line  
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3 747 of best fit with 95% confidence interval shaded in grey. Black bars indicate median,  
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5 748 within IQR (interquartile range) boxes for EuroImmuno/Roche/Abbott value. Red  
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8 749 triangles indicate outliers, based on  $1.5 \times$  IQR (interquartile range).  
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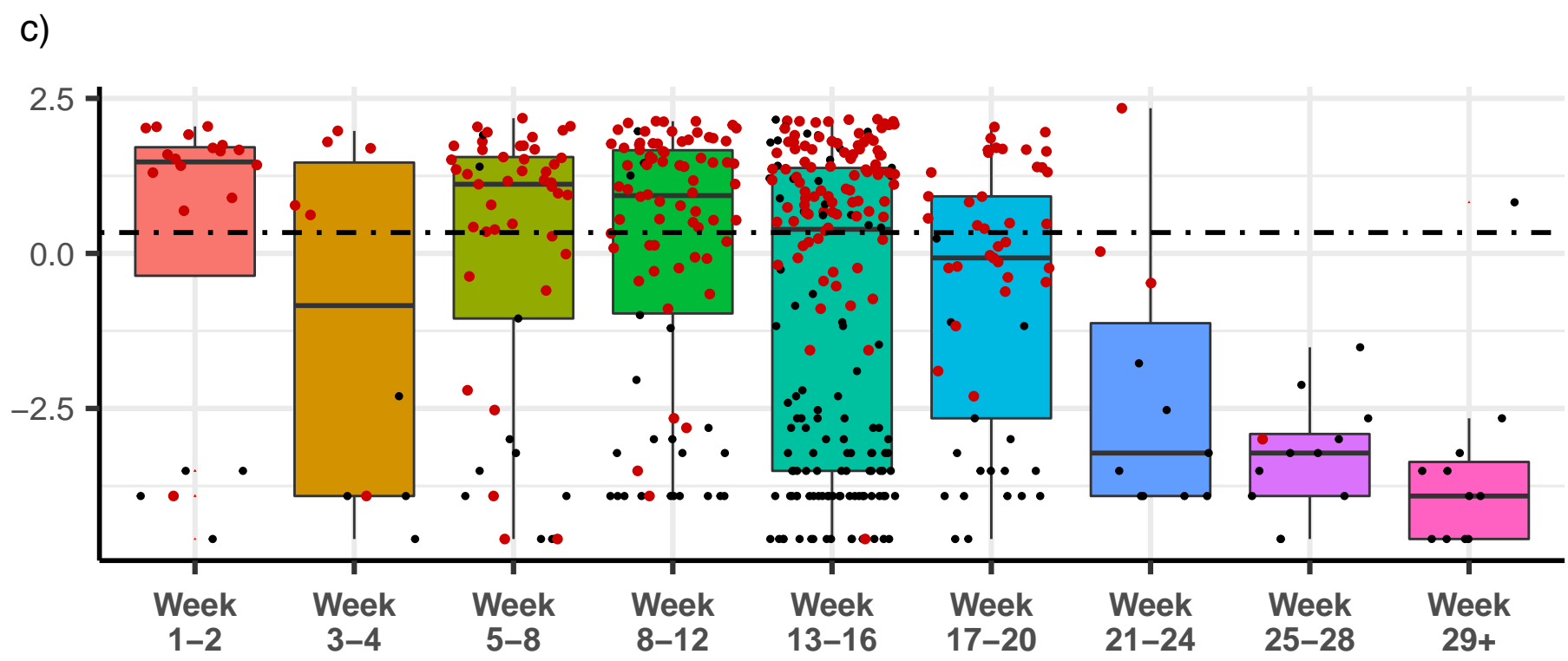
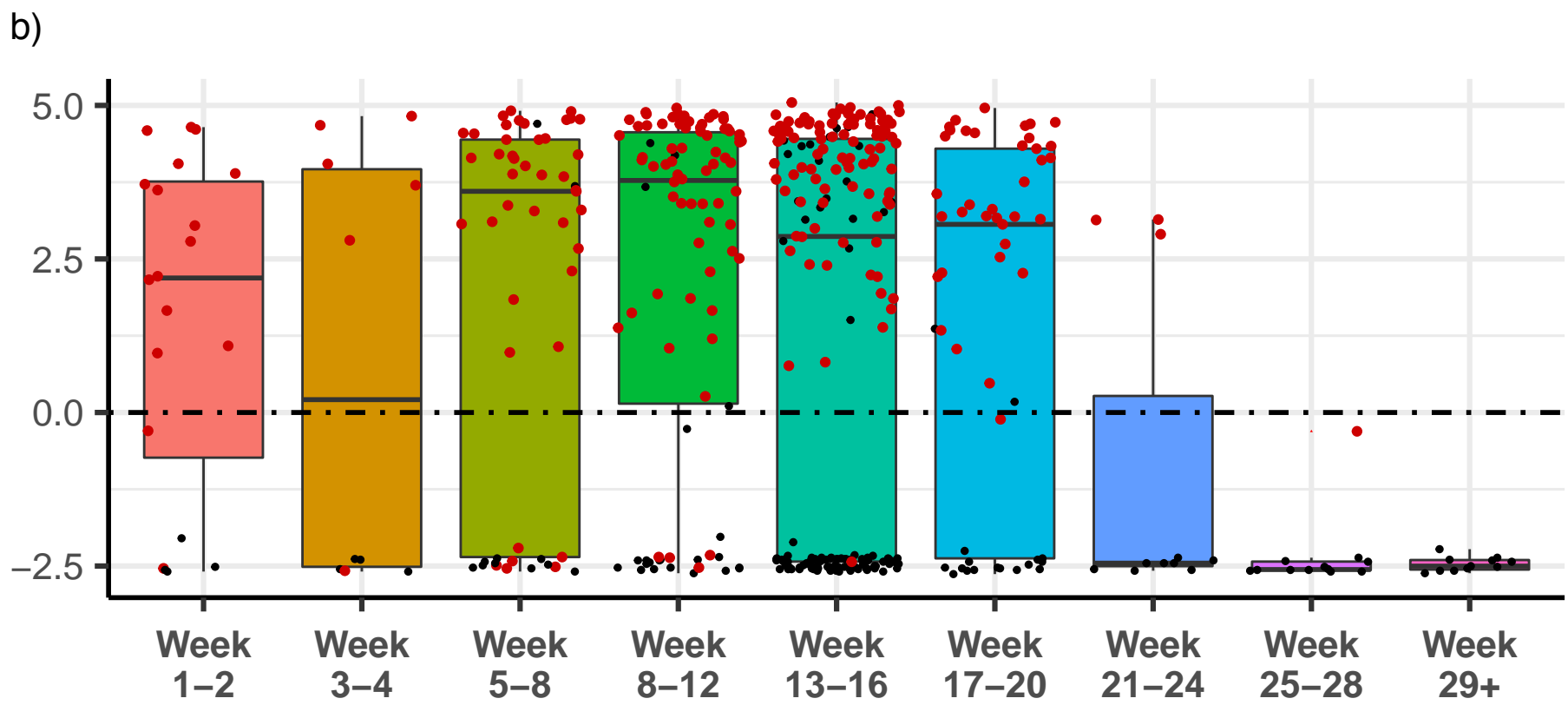
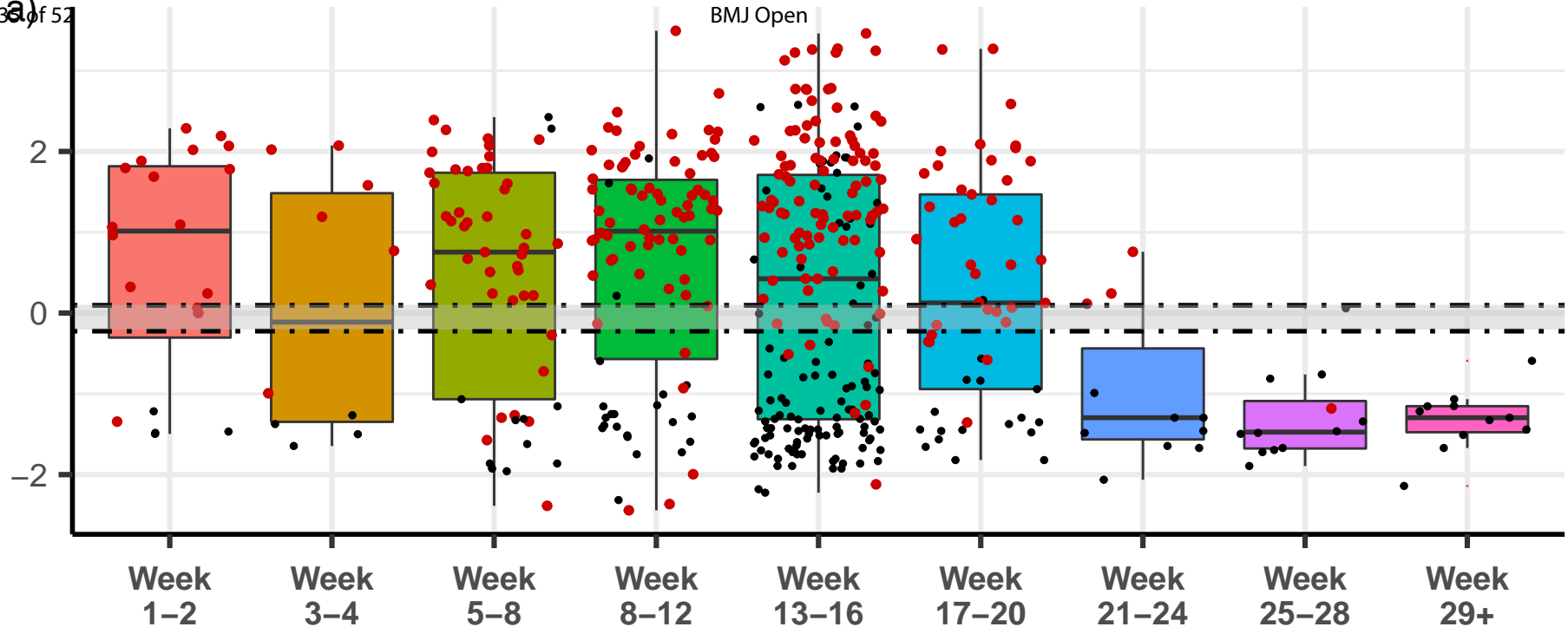


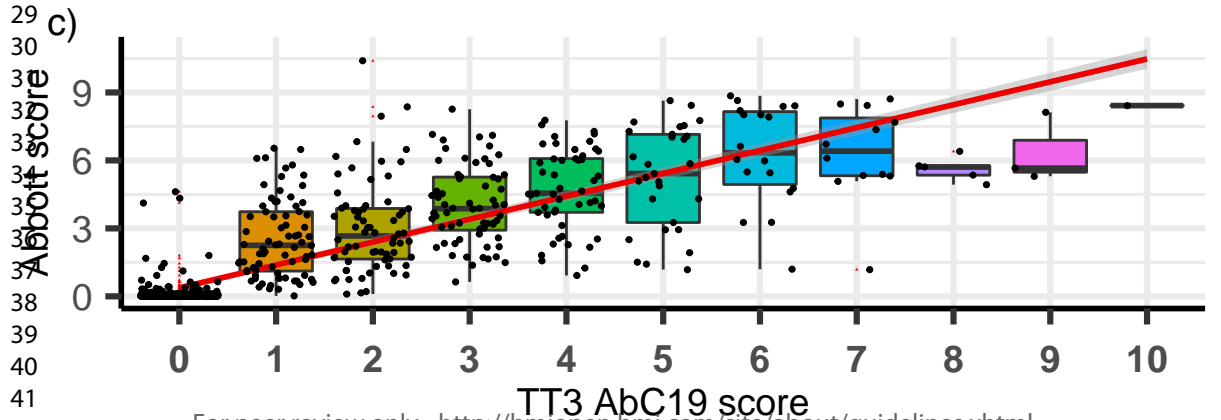
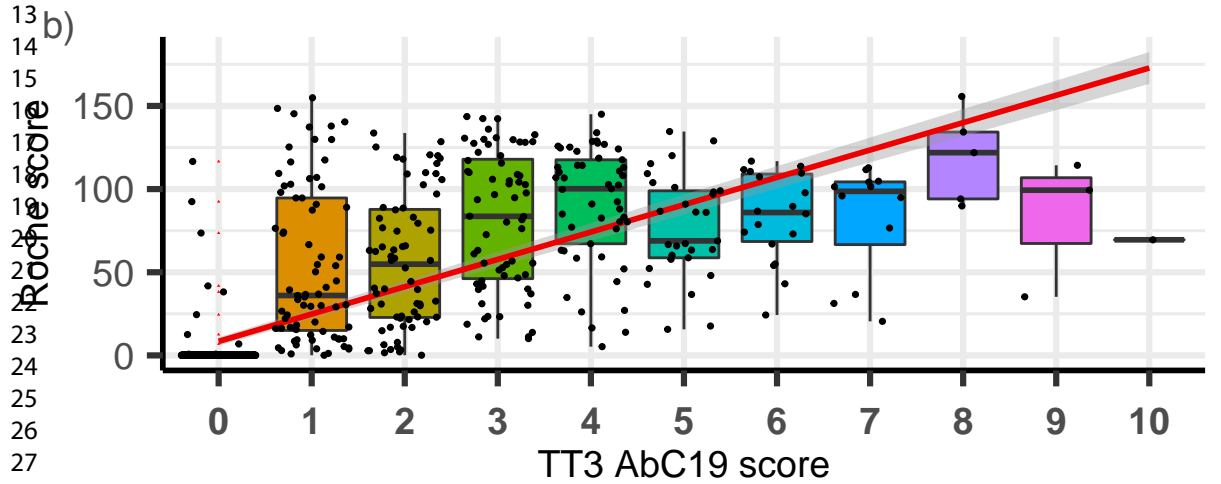
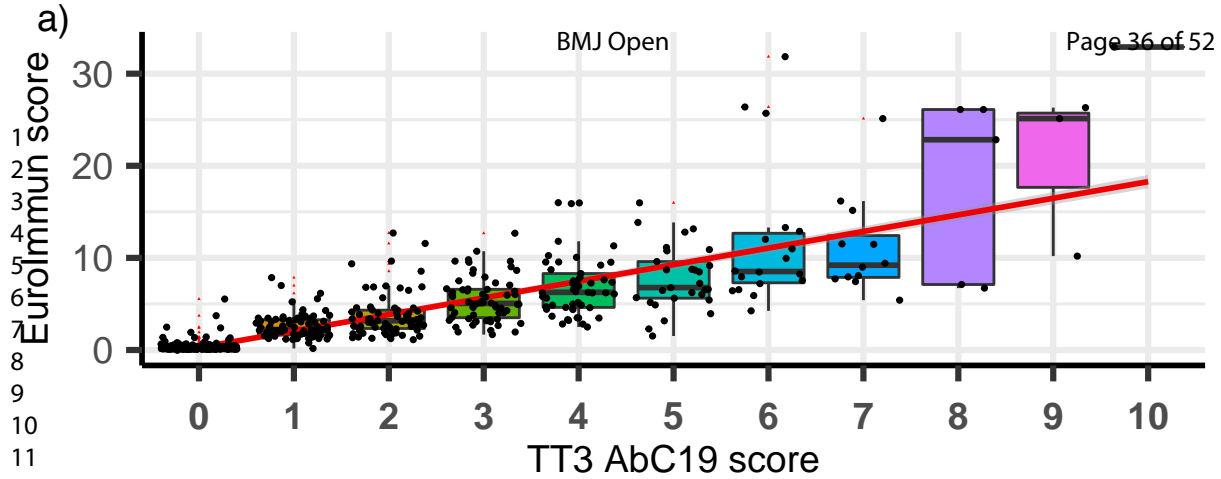
- Category**
- Both negative
  - Both positive
  - Disagreement
  - EuroImmun borderline

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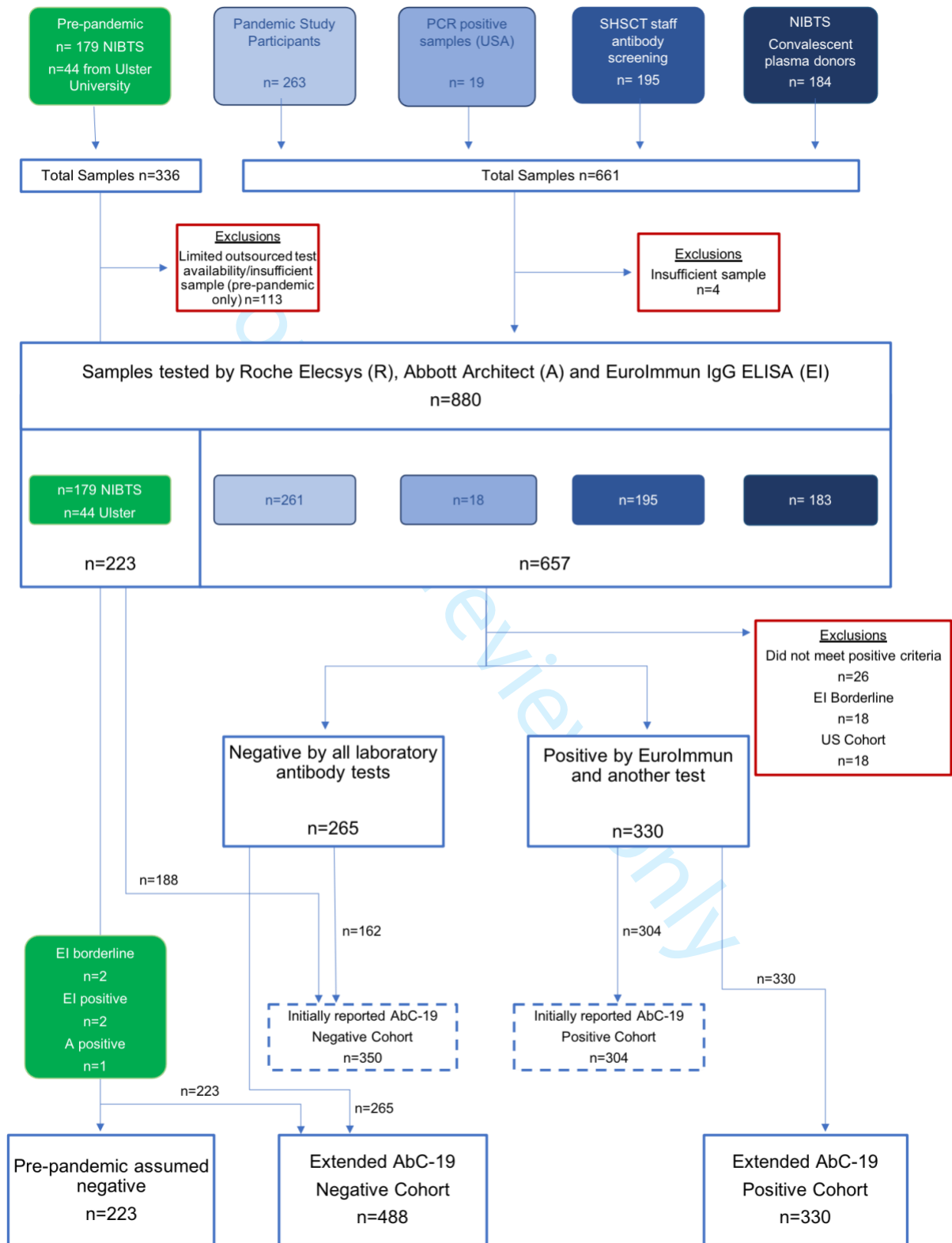
Fit lines, LOESS with 95% CI  
Vertical lines mark Abbott test range 0.25–1.4

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Supplementary Materials



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3 **Figure S1: Flow of participant plasma samples through cross-sectional study.**

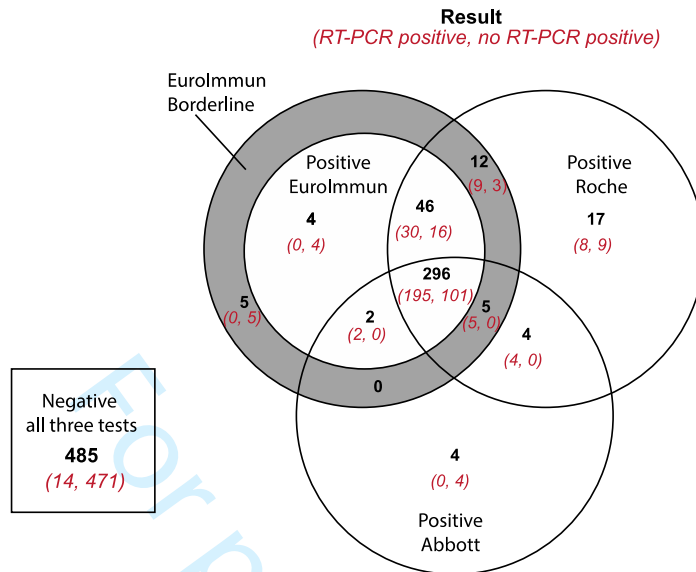
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5 All available samples from participants within each cohort, and the included and  
6 excluded samples at all stages. Freeze thaw cycles were closely monitored for all  
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8 excluded samples at all stages. Freeze thaw cycles were closely monitored for all  
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10 sample aliquots. Pre-pandemic samples taken forward for Roche, Abbott and  
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12 EuroImmun testing were selected based on aliquot volume and availability.  
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45 **Figure S2: Visual Score card for quantitative interpretation of AbC-19 LFIA test**  
46 **bands.** A scale of 0 (not pictured, negative-no test line visible) to 10 (positive-  
47 strongest test line). Any LFIA scoring 1 or above was classified as positive.  
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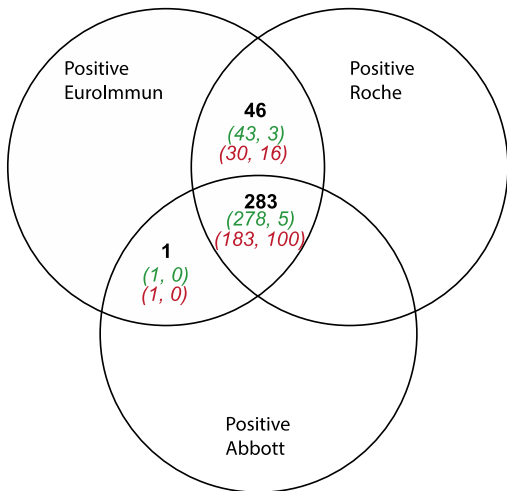
a)

### Laboratory immunoassays (n=880)



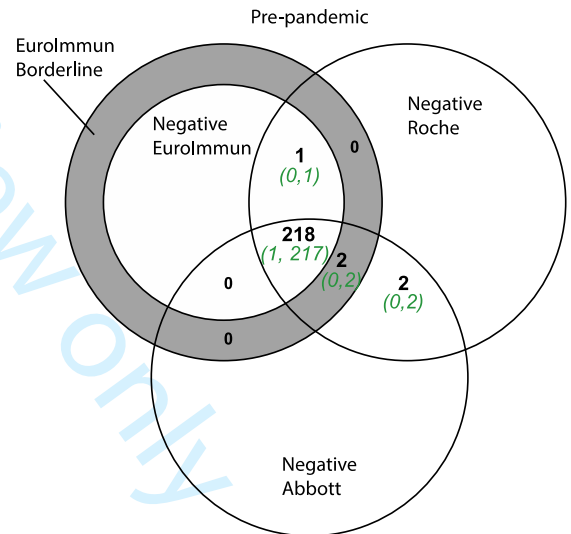
### b) Positive cohort (n=330)

**Result**  
*(AbC-19 positive, AbC-19 negative)*  
*(RT-PCR positive, no RT-PCR positive)*

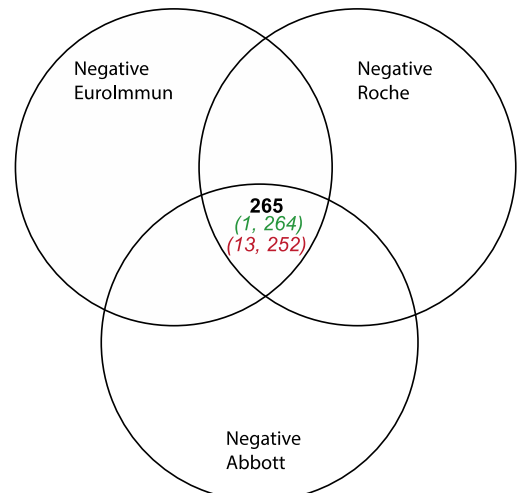


### c) Negative cohort (n=488)

**Result**  
*(AbC-19 positive, AbC-19 negative)*  
*(RT-PCR positive, no RT-PCR positive)*



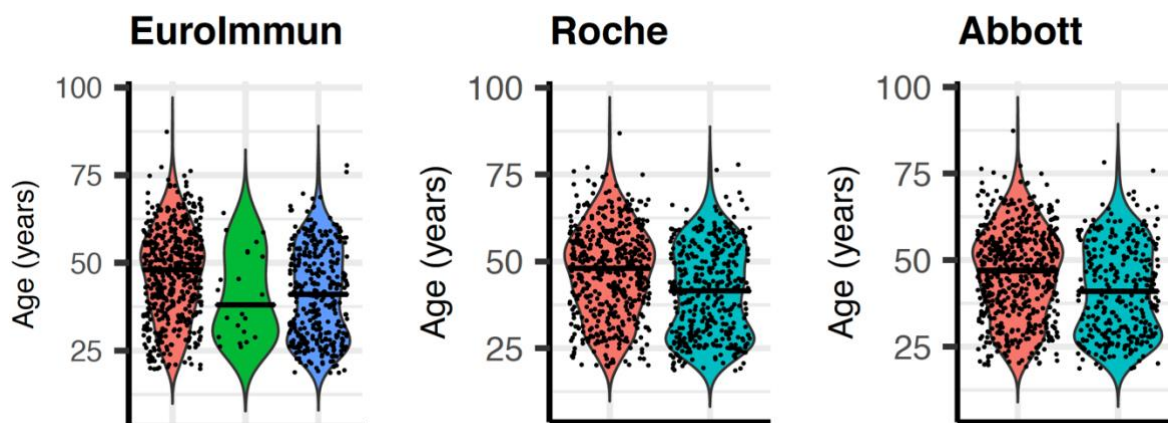
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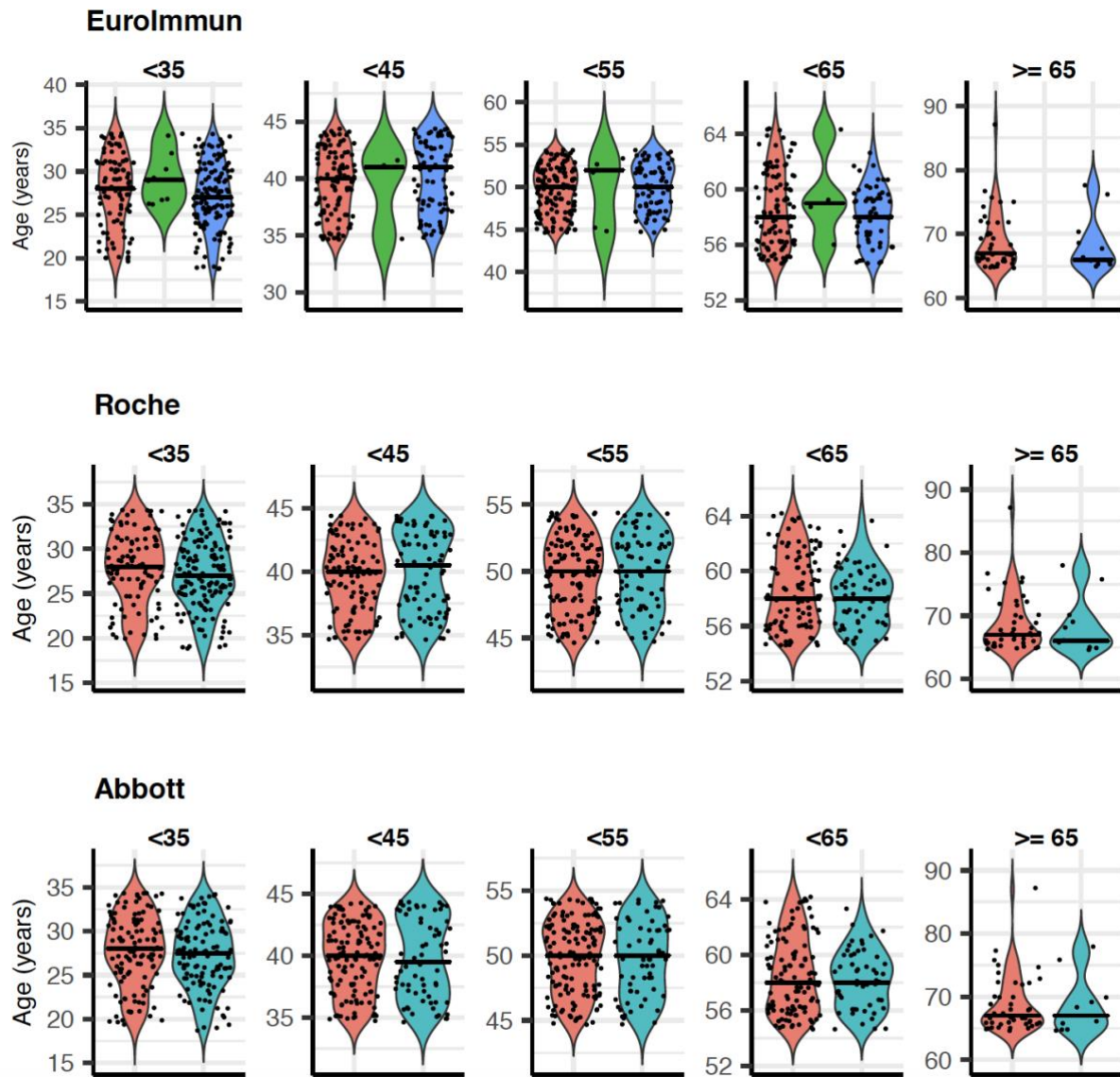
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3 **Figure S3: Venn diagrams demonstrating result overlap between laboratory**  
4 **assays in a) the initial immunoassay cohort (n=880), b) the positive and c)**  
5 **negative cohorts assessed with AbC-19 TT3.** Result in each circle overlap in bold,  
6 (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below. Where AbC-  
7 19 was analysed, (AbC-19 positive, AbC-19 negative) denoted in green.  
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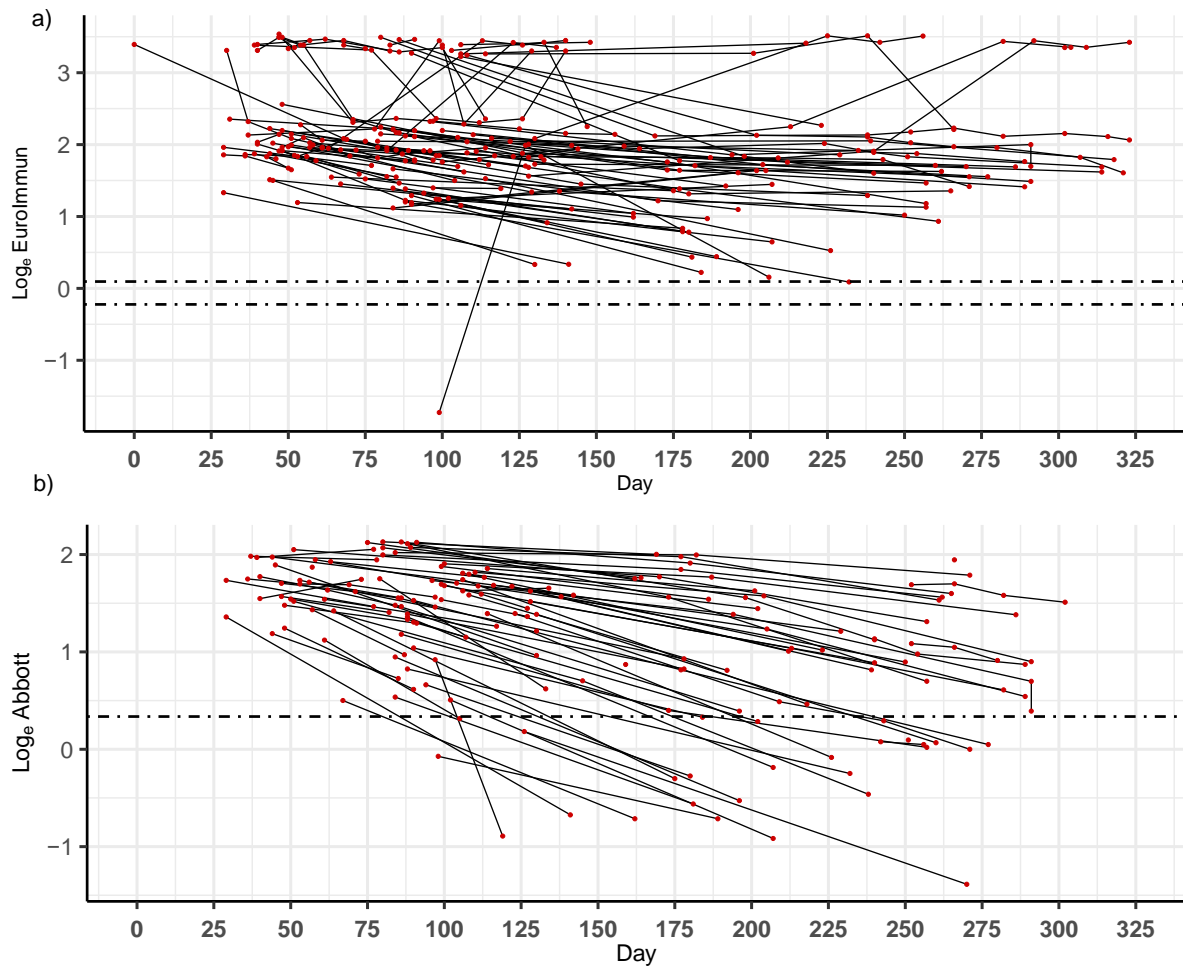
**Figure S4: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.**

The above graphs allow comparison of the distributions and probability density of ages for EuroImmune, 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.

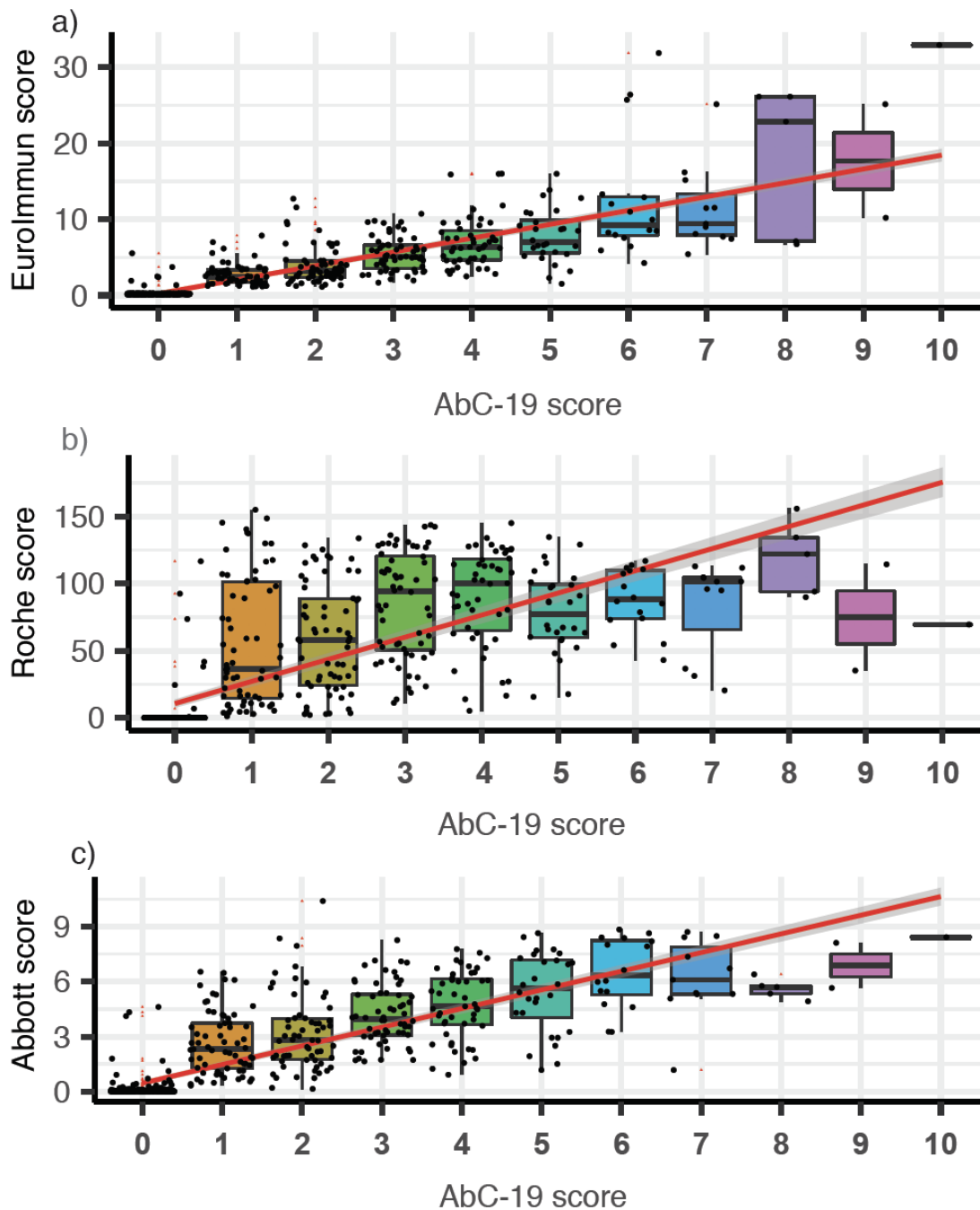


**Figure S5: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.**

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).

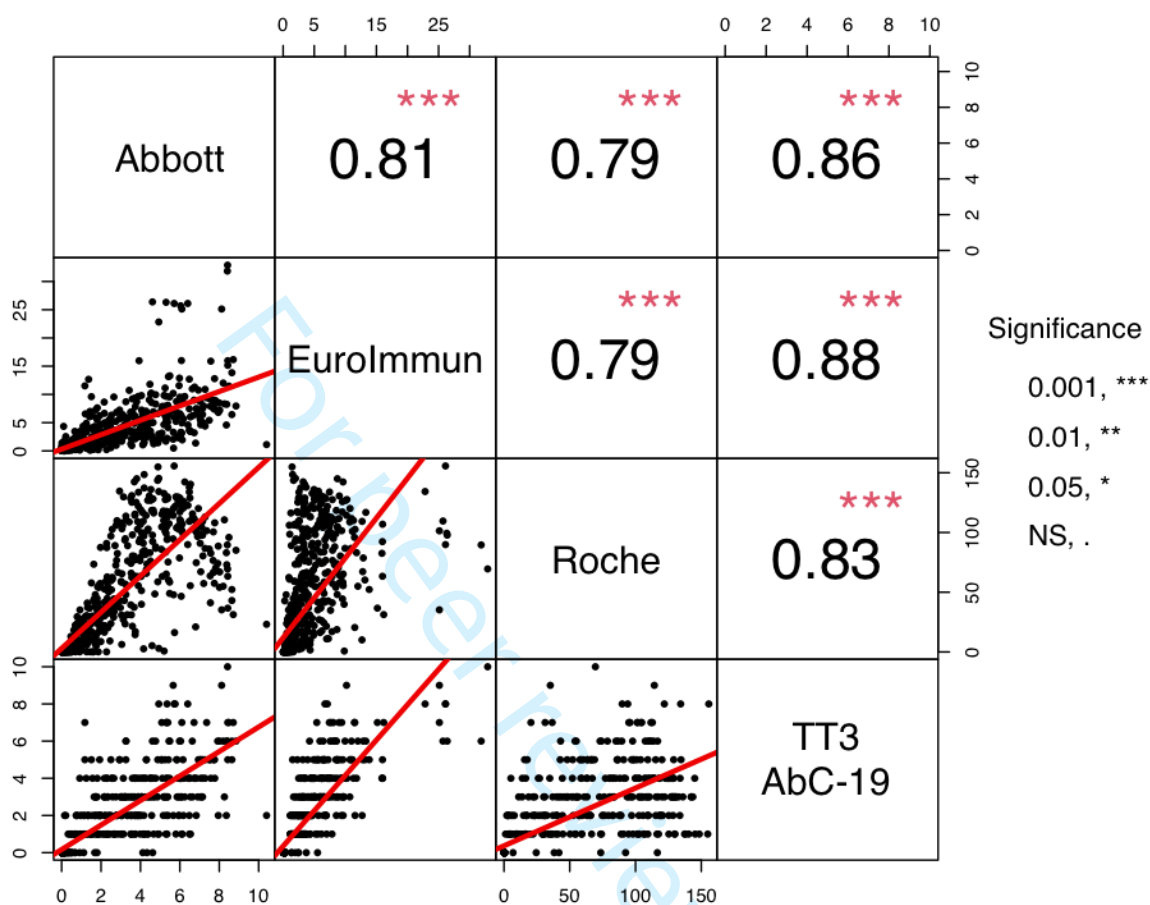


**Figure S6: Longitudinal analysis of convalescent plasma donor sequential samples (2-9 samples per individual) by a) EuroImmuno ELISA or b) Abbott immunoassay.** a) n=101 individuals, grey shading indicates borderline region, upper dotted line indicates positivity threshold (1.1), lower dotted line 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.

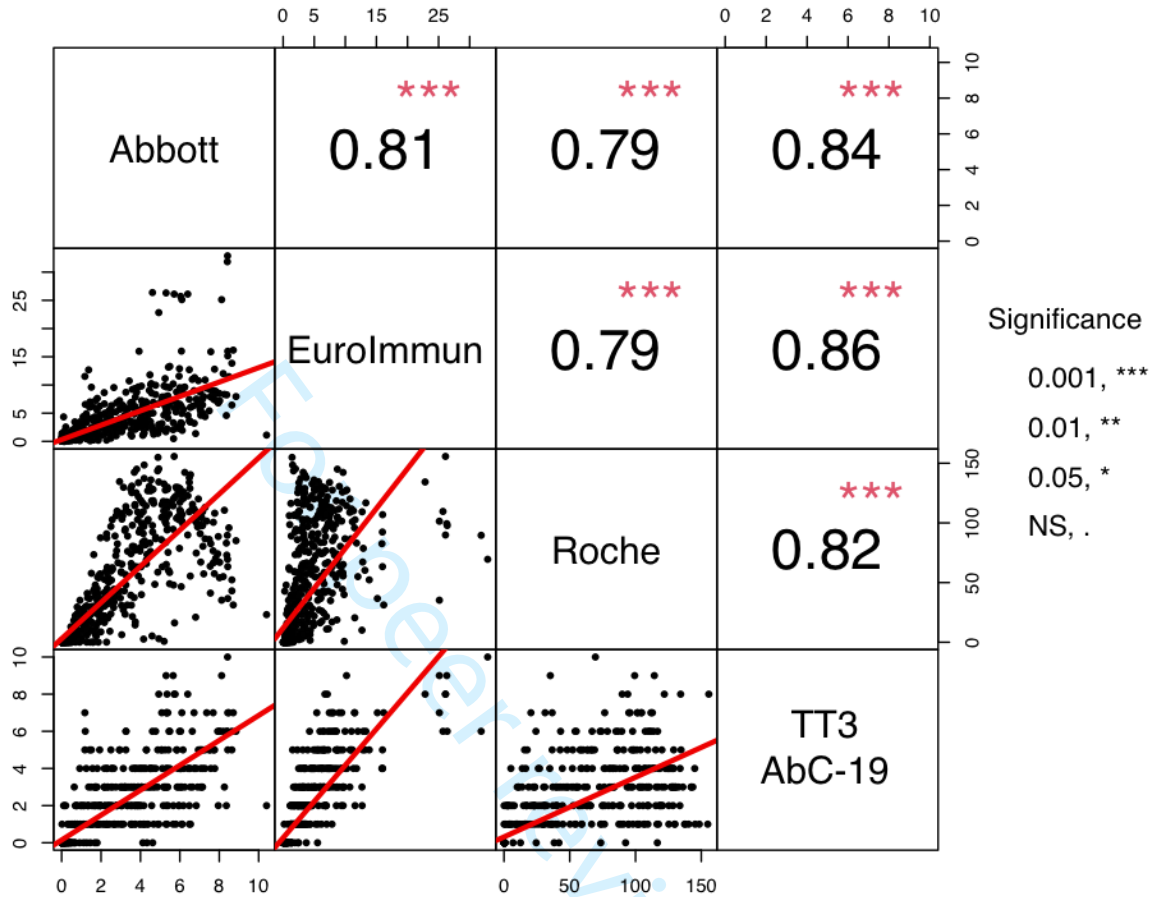


Linear fit line with 95% CI

**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  $1.5 \times$  IQR (interquartile range).

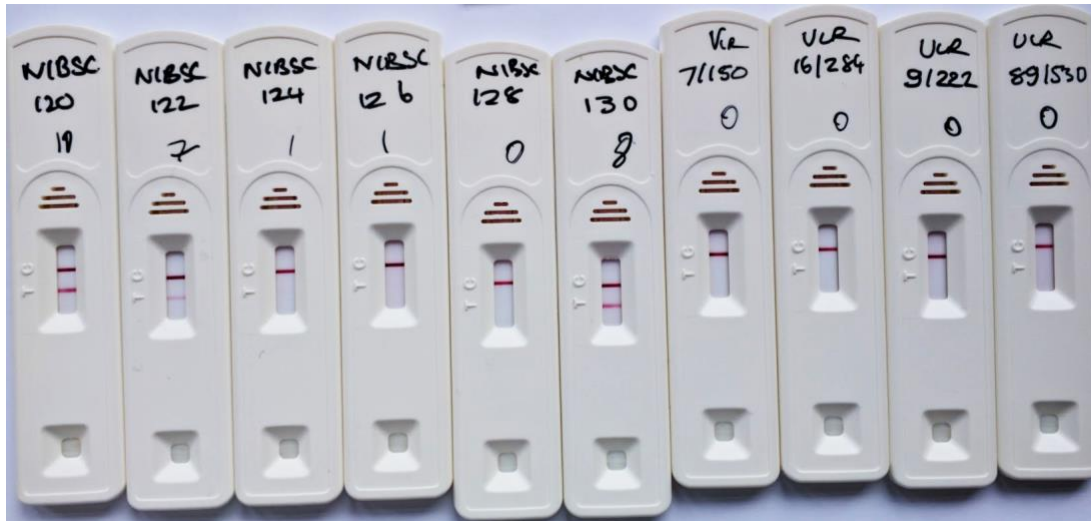


**Figure S8: Correlation matrix between Abbott, EuroImmun, Roche and initially reported AbC-19 cohort (n=654) quantitative output values for SARS-CoV-2 antibody levels.** Strong correlations are observed between all immunoassays. The level of significance was set at  $p < 0.05$ . All immunoassays were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



**Figure S9: Correlation matrix between Abbott, EuroImmuno, 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 LFAs 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
<b>EuroImmun ELISA</b>	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
<b>Roche Elecsys immunoassay</b>	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
<b>Abbott Architect SARS-CoV-2</b>	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
<b>3 AbC-19</b>	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%)
Eurolmmun	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** LFIA 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.

EuroImmuno Anti-SARS-CoV-2 ELISA-IgG (EuroImmuno, 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 EuroImmuno calibrator, with a score of < 0.8 determined negative,  $\geq 0.8$  to <1.1 borderline and  $\geq 1.1$  positive. For samples provided by NIBTS, EuroImmuno 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  $\geq 1.0$  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

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2  
3 20/B764-01). Results are reported by dividing the sample result by the calibrator result.  
4  
5 The result unit for the SARS-CoV-2 IgG assay is Index (Sample/Calibrator). A ratio of  
6  
7 < 1.4 is determined negative and  $\geq 1.4$  is determined positive.  
8  
9

### 10 11 12 *Analytical specificity and sensitivity assessment* 13

14 Four virology samples (H5N1 influenza serology 7/150, RSV serology 16/284,  
15  
16 Influenza B 9/222 and Bordetella Pertusis 89/530) were obtained from NIBSC  
17  
18 (National Institute for Biological Standards, Herts, UK). An additional 30 serology  
19  
20 samples from known virus infections were a kind gift from Sugentech, Seoul, Korea.  
21  
22 15 of these virology samples were obtained from Trina (Trina Bioreactives AG,  
23  
24 Switzerland) from 5 different individuals per virus (Influenza A IgG, Influenza B IgG  
25  
26 and RSV IgG). A further 15 of these virology samples were obtained from AbBaltris,  
27  
28 Kent from 5 different individuals per virus (Haemophilus Influenza IgG, Seasonal  
29  
30 Coronavirus NL63 and 229E Seasonal Coronavirus). All these serology samples  
31  
32 alongside a panel of 6 external standard research reagents (Table S4; NIBSC; Cat:  
33  
34 20/118 and 20/130) were assessed on the TT3 AbC-19 LFIA to confirm analytical  
35  
36 specificity and sensitivity.  
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Section & Topic	No	Item	Reported on page #
<b>TITLE OR ABSTRACT</b>			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
<b>ABSTRACT</b>			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
<b>INTRODUCTION</b>			
	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
<b>METHODS</b>			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
<i>Participants</i>	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
<i>Test methods</i>	9	Whether participants formed a consecutive, random or convenience series	6
	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
<i>Analysis</i>	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
<b>RESULTS</b>			
<i>Participants</i>	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
<i>Test results</i>	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
<b>DISCUSSION</b>			
	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
<b>OTHER INFORMATION</b>			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents

For peer review only

# 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
<b>Primary Subject Heading:</b>	Infectious diseases
<b>Secondary Subject Heading:</b>	Immunology (including allergy)
<b>Keywords:</b>	COVID-19, Molecular diagnostics < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES

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3 1 **Evaluation of the IgG antibody response to SARS CoV-2 infection and**  
4 2 **performance of a lateral flow immunoassay: cross-sectional and longitudinal**  
5 3 **analysis over 11 months**  
6 4

7 4  
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28 23  
29 23

1  
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3 **37 Abstract**  
4

5 **38 Objective**  
6

7 To evaluate the dynamics and longevity of the humoral immune response to SARS-  
8 CoV-2 infection and assess the performance of professional use of  
9 the UK-RTC AbC-19 Rapid Test lateral flow immunoassay (LFIA) for the target  
10 condition of SARS-CoV-2 spike protein IgG antibodies.  
11  
12  
13

14 **43 Design**  
15

16 Nationwide serological study.  
17

18 **45 Setting**  
19

20 Northern Ireland, UK, May 2020- February 2021.  
21

22 **47 Participants**  
23

24 Plasma samples were collected from a diverse cohort of individuals from the general  
25 public (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood  
26 donations and research studies (n=223) and through a convalescent plasma  
27 program (n=183). Plasma donors (n=101) were followed with sequential samples  
28 over 11 months post symptom onset.  
29  
30

31 **53 Main Outcome Measures**  
32

33 SARS-CoV-2 antibody levels in plasma samples using Roche Elecsys Anti-SARS-  
34 CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2  
35 ELISA immunoassays over time. UK-RTC AbC-19 LFIA sensitivity and specificity,  
36 estimated using a three-reference standard system to establish a characterised  
37 panel of 330 positive and 488 negative SARS-CoV-2 IgG samples.  
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39

40 **59 Results**  
41

42 We detected persistence of SARS-CoV-2 IgG antibodies for up to 10 months post  
43 infection, across a minimum of two laboratory immunoassays. On the known positive  
44 cohort, the UK-RTC AbC-19 lateral flow immunoassay showed a sensitivity of 97.58%  
45 (95.28%-98.95%) and on known negatives, showed specificity of 99.59% (98.53 %-  
46 99.95%).  
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51 **65 Conclusions**  
52

53 Through comprehensive analysis of a cohort of pre-pandemic and pandemic  
54 individuals, we show detectable levels of IgG antibodies, lasting over 46 weeks when  
55 assessed by EuroImmun ELISA, providing insight to antibody levels at later time points  
56 post-infection. We show good laboratory validation performance metrics for the AbC-  
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3 70 19 rapid test for SARS-CoV-2 spike protein IgG antibody detection in a laboratory-  
4 based setting.  
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9 73 **Strengths and Limitations**

10  
11 74 • Strength - This paper describes a non-clinical laboratory evaluation and  
12 comparison of the ability of three different immunoassays to detect SARS-CoV-  
13 75 2 antibodies in the same samples, detecting different subtypes of antibodies  
14 against different targets of the viral antigenic repertoire, that does not rely on  
15 76 PCR-positivity as definition of expected test outcome, to provide a panel of  
16 known antibody positive and antibody negative serology for evaluation of newly  
17 77 developed immunoassays.  
18  
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20 78  
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25 80  
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27 81  
28  
29 82 • Strength - This study demonstrates AbC-19 lateral flow point of care detection  
30 of IgG antibodies to the full trimeric spike protein of SARS-CoV-2 virus, the  
31 83 antibodies made in response to the vaccines used globally, in a large cohort of  
32 subjects, more than 10 months post infection, across a broad age range (18-  
33 84 78 years).  
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36 85  
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41 87  
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43 88 • Strength - This study assesses correlation between approved laboratory-based  
44 assays and the AbC-19 lateral flow point of care lateral flow test for the  
45 89 detection of SARS-CoV-2 antibodies in characterised cohorts of known positive  
46 and negative plasma samples in an evaluation conducted according to MHRA  
47 90 guidelines during a pandemic.  
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3 95 • Strength- Longitudinal data detecting IgG antibodies more than 10 months from  
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5 96 infection was collected as sequential samples over time through a convalescent  
6  
7 97 plasma donation program.  
8  
9  
10 98  
11  
12 99 • Limitation- This study was conducted in a standardised setting with very  
13  
14 100 experienced users on plasma characterised as positive or negative for the  
15  
16 101 presence of antibodies using a reference standard, alongside one other assay  
17  
18 102 which may introduce a possible spectrum bias and may not reflect the true  
19  
20 103 performance metrics of the assay evaluated when translated to real life  
21  
22 104 settings, using finger prick blood samples and in which pre-test probability  
23  
24 105 would impact greatly on positive and negative predictive values.  
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29 106

### 30 107 **Keywords**

31  
32 108 SARS-CoV-2, COVID-19, immunoassay, LFIA, pandemic, antibody assay  
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35 109

### 36 37 110 **Introduction**

38  
39 111 The World Health Organization declared a pandemic in March 2020 due to severe  
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41 112 acute respiratory syndrome coronavirus-2 (SARS-CoV-2), identified late 2019 in  
42  
43 113 Wuhan, China, causing Coronavirus Disease 2019 (COVID-19) disease (1,2).  
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46

47 114 A global race ensued to develop diagnostic assays, with the most common being viral  
48  
49 115 RNA detection (RT-qPCR assays), to detect acute infection(3). RT-qPCR assays are  
50  
51 116 labour and reagent intensive, limited by a short temporal window for positive diagnosis,  
52  
53 117 and exhibit potential for false negative results (4). Evidence suggests sensitivity of RT-  
54  
55 118 qPCR can be as low as 70% (5). False positive rates between 0.8- 4.0% have been  
56  
57 119 reported in the UK and are dependent on the Ct values accepted as indicating  
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3 120 infection, the number of SARS-CoV-2 genes analysed, and the proportion of  
4  
5 121 asymptomatic individuals tested (6,7). Lockdown measures and “flattening the curve”  
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7 122 strategies in the UK meant many infected individuals were instructed to self-isolate  
8  
9 123 and were not offered a diagnostic RT-qPCR, with much of the testing limited to patients  
10  
11 124 admitted to hospital, who perhaps reflect a more severely infected cohort.  
12  
13  
14 125 Consequently, a potentially large number of cases were unconfirmed or undetected  
15  
16  
17 126 (8).

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19  
20 127 The ability to accurately detect SARS-CoV-2 specific antibodies, which develop after  
21  
22 128 an immune response is evoked, is vital for building biobanks of convalescent sera for  
23  
24 129 treatment, monitoring immune response to infection alongside surveillance studies  
25  
26  
27 130 and assessing responses to vaccination programmes.

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29  
30 131 Commercial serology immunoassays are mostly laboratory-based and measure IgG  
31  
32 132 antibody levels in plasma or serum. Alternatively, lateral flow immunoassays (LFIA),  
33  
34 133 require a finger prick blood sample and can be used at point-of-care (POC) or in the  
35  
36 134 home; particularly important in the context of lockdown enforcement during the  
37  
38 135 pandemic. A limited number of laboratory-based chemiluminescence immunoassays  
39  
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41 136 are approved for use in the UK including the Roche Elecsys Anti-SARS-CoV-2  
42  
43 137 IgG/IgA/IgM against the SARS-CoV-2 Nucleocapsid antigenic region (Roche  
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45 138 Diagnostics, Basel, Switzerland) and the Abbott SARS-CoV-2 IgG assay against the  
46  
47  
48 139 same antigenic region (Abbott Diagnostics, Abbott Park, IL, USA).

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51 140 The complexities of the humoral immune response to SARS-CoV-2 is a much-  
52  
53 141 debated topic. In a US study, approximately one in 16 individuals lacked detectable  
54  
55 142 IgG antibodies up to 90 days post symptom onset, despite previous RT-PCR  
56  
57 143 confirmed infection (9). Patients who remain asymptomatic may mount a humoral  
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60 144 immune response which is short-lived, with detectable levels of antibody falling

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3 145 rapidly (10). This, alongside the lack of RT-PCR test availability across the UK has  
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5 146 hindered development of well characterised gold standard serology test for IgG  
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7 147 antibodies to SARS-CoV-2.

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9  
10 148 Herein, we describe the use of Roche and Abbott commercial immunoassays, as well  
11  
12 149 as the EuroImmun Anti-SARS-CoV-2 ELISA-IgG against the S1 domain of the spike  
13  
14 150 (antigenic) protein of SARS-CoV-2 (EuroImmun UK, London, UK) to characterise pre-  
15  
16 151 pandemic and pandemic COVID-19 blood samples (n=880) from within Northern  
17  
18 152 Ireland and report on longevity of IgG antibodies detected. Furthermore, we follow IgG  
19  
20 153 antibody levels in convalescent plasma donors (n=101 individuals) for up to 11  
21  
22 154 months. Currently, there is no gold standard assay for comparison, therefore we aimed  
23  
24 155 to establish a reference based on a positive COVID-19 antibody status. We present  
25  
26 156 results of a laboratory evaluation of the UK-RTC AbC-19 with a target condition of  
27  
28 157 antibodies against a cohort of 330 known IgG antibody positive samples according to  
29  
30 158 this 'positive by two' system and 488 negative samples (223 pre-pandemic assumed  
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32 159 negative and 265 known negative) for IgG to SARS-CoV-2.  
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160

## 161 **Methods**

162

### 163 **Participant samples**

164 The flow of participant samples is summarised in Figure S1. A small cohort (n=19) of  
165 anonymised plasma samples were obtained from a partner USA laboratory for initial  
166 protocol development only. All participants provided informed consent. An online  
167 recruitment strategy was employed, with the study advertised through internal Ulster  
168 University email, website and social media. A BBC Newsline feature providing the  
169 pandemic study email address also prompted interest from the general population.  
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3 170 The first 800 respondents who expressed interest were provided with an online patient  
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5 171 information sheet, consent form and health questionnaire and invited to register to  
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7 172 attend a clinic. Participants were eligible for the study if they were over 18 years of  
8  
9 173 age. Exclusion criteria included anyone with a blood disorder or contraindication to  
10  
11 174 giving a blood sample, or anyone currently exhibiting symptoms of COVID-19. To  
12  
13 175 enrich the cohort for samples potentially positive for SARS-CoV-2 IgG antibody,  
14  
15 176 further participants were invited if they had previously tested PCR positive or had the  
16  
17 177 distinctive symptom of loss of taste and smell. Blood sampling clinics were held at  
18  
19 178 locations around Northern Ireland between May and July 2020 resulting in collection  
20  
21 179 of 263 10ml EDTA plasma samples from 263 separate study participants. Additional  
22  
23 180 anonymised plasma samples were obtained from Southern Health and Social Care  
24  
25 181 Trust (SHSCT) Healthcare workers (n=195), and Northern Ireland Blood Transfusion  
26  
27 182 Service (NIBTS, n=184) through convalescent plasma programs. NIBTS convalescent  
28  
29 183 plasma samples continued to be collected throughout 2020-early 2021, with a total of  
30  
31 184 n=897 from n=676 individuals, including n=183 samples from the cross-sectional  
32  
33 185 cohort. Individuals from this program with a positive RT-PCR result and EuroImmun  
34  
35 186 starting value >6 were sequentially sampled over a period of up to 46 weeks resulting  
36  
37 187 in a cohort of n=101 individuals, n=296 samples (including n=47 individuals from the  
38  
39 188 cross-sectional cohort).

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49 190 Pre-pandemic samples (prior to June 2019, n=136) were obtained from Ulster  
50  
51 191 University ethics committee approved studies with ongoing consent and from NIBTS  
52  
53 192 (n= 200, more than 3 years old). Plasma samples were used at no more than 3 freeze-  
54  
55 193 thaw cycles for all analyses reported within this manuscript.

194

## 195 **Clinical information**

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

202

## 203 **Laboratory-based immunoassays**

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

206

## 207 **UK-RTC AbC-19 LFIA**

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

216

## 217 **Statistical analysis**

218 As per Daniel (11) a minimum sample size based on prevalence can be calculated

219 using the following formula:  $n = \frac{Z^2 P(1 - P)}{d^2}$ , where n = sample size, Z = Z statistic for a

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3 220 chosen level of confidence,  $P$  = estimated prevalence, and  $d$  = precision. Assuming  
4  
5 221 a prevalence of SARS-CoV-2 of 10% and a precision of 5%, we estimate that the  
6  
7 222 required sample size at 99% confidence ( $Z = 2.58$ ) to be 240 individuals. If the true  
8  
9 223 prevalence is lower, 5%, the estimated required sample size given a precision of  
10  
11 224 2.5% is 506 individuals. A minimum sample size of 200 known positives and 200  
12  
13 225 known negatives is given within MHRA guidelines for SARS-CoV-2 LFIA antibody  
14  
15 226 immunoassays(12).

16  
17 227 Statistical analysis was conducted in in R v 4.0.2(13). To assess discordance between  
18  
19 228 test results, data was first filtered to include individuals with an Abbott test result in the  
20  
21 229 range  $\geq 0.25$  &  $\leq 1.4$ , with a 2 x 2 contingency table produced that comprised all  
22  
23 230 possible combinations of [concordant|discordant] test results [within|outside of] this  
24  
25 231 range. A p-value was derived via a Pearson  $\chi^2$  test after 2000 p-value simulations via  
26  
27 232 the stats package.

28  
29 233 AbC-19 LFIA performance analyses were performed using MedCalc online (MedCalc  
30  
31 234 Software, Ostend, Belgium). ROC analysis was performed via the pROC package. To  
32  
33 235 compare test result (Positive|Negative) to age, a binary logistic regression model was  
34  
35 236 produced with test result as outcome – a p-value was then derived via  $\chi^2$  ANOVA. To  
36  
37 237 compare time against test result (encoded continuously), a linear regression was  
38  
39 238 performed. We calculated median per time-period and then converted these to log  
40  
41 239 [base 2] ratios against the positivity cut-off for each assay. All plots were generated  
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43 240 via ggplot2 or custom functions using base R(14).

44  
45 241

## 46 47 242 **Results**

48  
49 243 We analysed samples from a mixed cohort of individuals from the general public  
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51 244 (n=279), Northern Ireland healthcare workers (n=195), pre-pandemic blood donations  
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3 245 and research studies (n=223) and through a convalescent plasma program (n=183).  
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5 246 Antibody levels in plasma from these 880 individuals were assessed using the three  
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7 247 SARS-CoV-2 immunoassays; EuroImmun IgG, Roche Elecsys IgG/IgM/IgA and  
8  
9 248 Abbott Architect IgG (Table S1, Figure S3). This included a cohort of 223 pre-  
10  
11 249 pandemic plasma samples collected and stored during 2017 to end of May 2019 to  
12  
13 250 determine assay specificity. Of the 657 participants whose samples were collected  
14  
15 251 during the pandemic, 267 (40.64%) previously tested RT-PCR positive with a range of  
16  
17 252 7-173 days since diagnosis. A total of 225 participants gave time since self-reported  
18  
19 253 COVID-19 symptoms, with a range of 5-233 days from symptom onset, whilst 195 had  
20  
21 254 no symptom or PCR data available. Samples collected in 2020 (n=657) ranged from  
22  
23 255 19-78 years of age with a median (IQR) of 43 years ( $\pm 22$ ), and n=454 were female  
24  
25 256 and n=200 male (n=3, not disclosed). Pre-pandemic samples (n=223) ranged from 20-  
26  
27 257 87 years of age with median (IQR) of 50 years ( $\pm 20$ ) and consisted of n=88 female  
28  
29 258 and n=135 male.

### 30 31 32 33 34 35 259 *Laboratory based antibody immunoassays*

36  
37 260 A positive result for antibody on one or more of the three laboratory immunoassays  
38  
39 261 was recorded for 385/657 (58.6%) participants who provided a sample during the  
40  
41 262 pandemic. By EuroImmun ELISA, 346 were positive, 20 borderline and 291 were  
42  
43 263 negative. The Roche assay detected 380 positive and 277 negative, whilst Abbott  
44  
45 264 determined 310 positive and 347 negative (Table S2, Figure S3). The median age  
46  
47 265 across all age groups combined was lower for participants testing positive across each  
48  
49 266 of the immunoassays (median [sd] for positive versus negative, respectively:  
50  
51 267 EuroImmun, 41 [13.16] vs 48 [12.95]; Roche, 42 [13.08] vs 48 [13.00]; Abbott, 41  
52  
53 268 [13.18] vs 47 [13.09]). (Figure S4,  $p < 0.0001$ ). When segregated by age group,  
54  
55 269 however, differences were less apparent in certain groups (Figure S5). Excluding the  
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3 270 pre-pandemic cohort, this gap reduced but remained statistically significant  
4  
5 271 EuroImm, 41 [13.18] vs 45 [12.49]; Roche, 42 [13.15] vs 45 [12.49]; Abbott, 41  
6  
7 [13.26] vs 44 [12.63]) ( $p < 0.01$ ) (median [sd] for positive versus negative). Of note, out  
8  
9 of 267 individuals with a previous positive RT-PCR result for SARS-CoV-2 viral RNA,  
10  
11 273 14 (5.2%, Figure S3a) did not show detectable antibodies by any of the three  
12  
13 274 immunoassays, with no association found with age, gender or time between test and  
14  
15 275 blood draw (data not shown).  
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19  
20 277 The three commercial laboratory immunoassays provide a ratio value that increases  
21  
22 278 with IgG antibody titre. When correlation between these values is assessed, good  
23  
24 279 overall agreement is observed between the three immunoassays (Figure 1a-c, Figure  
25  
26 280 S5). As highlighted by Rosadas *et al.*, we also see significant disagreement in the  
27  
28 281 Abbott 0.25-1.4 range when compared to EuroImm and Roche (Figure 1a,b; chi-  
29  
30 square p-values: EuroImm vs Abbott,  $p < 0.001$ ; Roche vs Abbott,  $p < 0.001$ )(15).  
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#### 35 36 37 284 *Duration of humoral response to SARS-CoV-2*

38  
39 285 In a cross-sectional analysis of antibodies over time, we found IgG antibodies could  
40  
41 286 still be detected in individuals (excluding pre-pandemic) across all three  
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43 287 immunoassays used up to week 20 (day 140) (Figure 2). We note a statistically  
44  
45 288 significant decrease in signal with respect to time across each assay (p-value  
46  
47 [estimate slope]): EuroImm,  $p = 0.028$ [-0.823]; Roche,  $p = 0.002$ [-0.125]; Abbott,  
48  
49 289  $p < 0.0001$ [-3.673]. These remained statistically significant after adjustment for age.  
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51 290  
52  
53 291 Antibody levels (expressed as a ratio of median result per timepoint divided by  
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55 292 positivity cut off; Table 1) peaked at Week 1-2 for EuroImm (1.33) and Abbott  
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57 293 (1.64), though reached highest levels at Week 8-12 when measured by Roche  
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59 294 (5.45). By week 21-24, median score for all tests had dropped below the positivity

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3 295 cut off, though a small number of samples remained above the positive cut off at  
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5 296 these later timepoints (Figure 2).  
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7 297 Samples from the NIBTS convalescent plasma program continued to be collected  
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9 298 throughout 2020-early 2021. A total of n= 897 samples from n=676 individuals were  
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11 299 collected, 744/883 tested by EuroImmun were positive (>1.1, with values range of  
12  
13 300 0.051-34.361), 556/749 tested by Abbott were positive (>1.4, with values ranging  
14  
15 301 from 0.01-8.85). Individuals with a positive RT-PCR result and a EuroImmun result  
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17 302 >6 were sequentially sampled (with median 3, range 2-9 samples per individual) and  
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19 303 analysed by both EuroImmun (n=101 individuals), and Abbott immunoassays (n=75  
20  
21 304 individuals). Median age (IQR) for this cohort is 51 years ( $\pm 21$ ) with a range from 18-  
22  
23 305 70 years and n=27 female, n=74 male. Longitudinal analysis shows persistence of  
24  
25 306 detectable IgG antibodies until up to 302 days (43 weeks) by Abbott immunoassay  
26  
27 307 (at which point this assay was discontinued at NIBTS) and 323 days (46 weeks) by  
28  
29 308 EuroImmun ELISA, with a gradual decline over time (Figure S6). None of the  
30  
31 309 individuals who were initially positive by Euroimmun SARS-CoV-2 S1 IgG assay  
32  
33 310 dropped to below the EuroImmun positivity threshold (>1.1) over the course of the  
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35 311 follow-up while 26 who were initially positive by Abbott SARS-CoV-2 NP IgG fell  
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37 312 below the Abbott threshold (>1.4).  
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#### 314 *UK-RTC AbC-19*

315 Using the commercial immunoassays described we established a well characterised  
316 serology sample set of 'known positive' and 'known negative' for IgG antibodies to  
317 SARS-CoV-2 to evaluate performance metrics for the UK-RTC AbC-19 Rapid LFIA.  
318 AbC-19 detects IgG antibodies against the spike protein antigen, so we therefore  
319 required all samples to be positive by the EuroImmun SARS-CoV-2 IgG ELISA, which

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3 320 likewise detects antibodies against the S1 domain (16). To develop this characterised  
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5 321 cohort, samples were also required to be positive by a second immunoassay (Roche  
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7 322 or Abbott). To analyse specificity of the AbC-19 LFIA for detection of SARS-CoV-2 IgG  
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9 323 antibody, we assessed 350 plasma samples from participants classed as 'known  
10  
11 324 negative for SARS-CoV-2 IgG antibody' on the AbC-19 LFIA. All samples were from  
12  
13 325 individuals confirmed to be negative across all three laboratory assays (Roche,  
14  
15 326 EuroImmun, Abbott). Using these positive n=304 and negative n=350 antibody  
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17 327 cohorts, we determined a sensitivity for detecting SARS-CoV-2 IgG antibody of  
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19 328 97.70% (95% CI; 95.31%-99.07%) and specificity of 100% (98.95%-100.00%) for the  
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21 329 AbC-19 LFIA (Table 2).

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26 330 Given a recent report of lower specificity in the AbC-19 LFIA (17) and the possibility  
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28 331 of introducing sample bias, we revised our inclusion criteria for the negative cohort.  
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30 332 For the pre-pandemic cohort, we included samples from all 223 individuals,  
31  
32 333 regardless of results on other laboratory immunoassays. When this assumed  
33  
34 334 negative pre-pandemic cohort was used for laboratory evaluation for target condition  
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36 335 of antibodies, we observed a specificity of 99.55% (97.53% to 99.99%, Table 2). We  
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38 336 expanded the negative cohort to include all samples that matched our criteria  
39  
40 337 (samples collected during the pandemic to be negative by all three laboratory assays  
41  
42 338 and all pre-pandemic samples regardless of other immunoassay results). The  
43  
44 339 specificity observed on this extended negative cohort of 488 samples was 99.59%  
45  
46 340 (98.53% to 99.95%, Table 2). For sensitivity analysis on a positive cohort (samples  
47  
48 341 positive by EuroImmun and one other test), we were able to analyse all samples  
49  
50 342 previously untested due to limited testing capacity and tested a positive cohort of 330  
51  
52 343 samples giving a sensitivity of 97.58% (95.28% to 98.95%, Table 2). When we  
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54 344 sorted samples analysed in both negative (n=488) and positive cohorts (n=330) by  
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3 345 RT-PCR status and assessed AbC-19 LFIA sensitivity by including only those that  
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5 346 were RT-PCR positive (n=227), the test showed a sensitivity of 92.07% (87.76%-  
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7 347 95.23%, Table S3, Figure S3b). However, of the n=18 RT-PCR positive individuals  
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9 348 negative for IgG antibodies by AbC-19, n=12 showed no detectable antibodies by all  
10  
11 349 three laboratory assays (EuroImmun, Roche or Abbott), suggesting that antibodies  
12  
13 350 are not present in those samples (Figure S3c).  
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19 352 When used as intended by the public, the AbC-19 LFIA provides binary  
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21 353 positive/negative results. However, when assessing LFIA in the laboratory, each test  
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23 354 line was scored against a scorecard by three independent researchers (0 negative, 1-  
24  
25 355 10 positive; Figure S2). When compared to quantitative outputs from the Abbott,  
26  
27 356 EuroImmun and Roche assays, the AbC-19 LFIA shows good correlation (Abbott  
28  
29 357  $r=0.84$  [ $p<0.001$ ]; EuroImmun  $r=0.86$  [ $p<0.001$ ]; Roche  $r=0.82$  [ $p<0.001$ ]; Figure 3,  
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31 358 Figure S7-Figure S9).  
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### 360 *Analytical specificity and sensitivity of AbC-19 LFIA*

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



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3 369 Serological antibody immunoassays are an important tool in helping combat the  
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5 370 SARS-CoV-2 pandemic. The duration of the humoral immune response is of  
6  
7 371 particular importance, to inform an individual's protection following both natural  
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9 372 infection and vaccination. Using a large cohort of individuals across a wide age  
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11 373 range (18-78 years), we assessed antibody levels across up to three laboratory  
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13 374 immunoassays perform a cross-sectional and longitudinal analysis over time. Our  
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15 375 results show strong correlation between all three immunoassays, with shortcomings  
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17 376 in the Abbott system output 0.25-1.4 range, as described previously, suggesting an  
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19 377 overestimated positive cut-off (Figure 1) (15).  
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26 379 Longitudinal studies on SARS-CoV-1 convalescent patients suggests that detectable  
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28 380 IgG can still be present as long as 2 years after infection (18). There are conflicting  
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30 381 reports of the longevity of the humoral response to SARS-CoV-2 infection which  
31  
32 382 differ in the make-up of the cohort studied, the assays used, and the length of time  
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34 383 since symptom onset. The longevity of IgG antibodies to both spike and  
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36 384 nucleocapsid protein more than 10 months after RT PCR positive status (and  
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38 385 beyond in a small number of samples, Figure 2, Figure S6) is consistent with that  
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40 386 observed in other recent studies(19–21). In this study, samples were collected  
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42 387 through a convalescent plasma program (Figure S6), with individuals selected for  
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44 388 sequential plasma donation based on an initial high EuroImmun assay score. In  
45  
46 389 contrast to the time series analysis of healthcare workers recruited prospectively by  
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48 390 Manisty *et al.*, we observed no cases where Euroimmun ELISA-measured anti-Spike  
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50 391 antibody levels fell below threshold, whilst a large number of Abbott measured anti-  
51  
52 392 Nucleocapsid antibody levels dropped below the positivity threshold (34.7% 26/75).  
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54 393 However, this may be an overestimate given the shortcomings of the Abbott assay  
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3 394 described above (Figure 1) (22). In a similar longitudinal study of 51 symptomatic  
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5 395 participants, Dan *et al.* estimated that half-life ( $t_{1/2}$ ) for IgG-Spike (103 days) was  
6  
7 396 longer than that for IgG-Nucleocapsid (68 days), although with a considerable  
8  
9 397 overlap of 95% confidence intervals (23).

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11  
12 398 In our more diverse cross-sectional cohort, we also note a statistically significant  
13  
14 399 decline over time but levels remain detectable at 140 days (Figure 2). We note that  
15  
16 400 IgG levels reach their peak (Roche ratio 5.45 times threshold cut-off) as late as  
17  
18 401 Week 8-12 from first symptoms or a viral RNA RT-PCR positive result, though this  
19  
20 402 may be an artefact of lower number of participants at earlier timepoints (Table 1).  
21  
22 403 Robust antibody responses are produced in our cohorts across a wide age range  
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24 404 (18-78 years old, Figure 2, Figure S6). We detect a slightly but significantly lower  
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26 405 median age of participants testing positive (Figure S4); however, this is likely be due  
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28 406 to cohort characteristics and not a true reflection of the population or indication of  
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30 407 test performance.  
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409 A difficulty faced in validation of antibody diagnostic assays has been access to  
410 samples with known SARS-CoV-2 antibody status. As previously described, there is  
411 no clear gold standard reference against which to assess SARS-CoV-2  
412 immunoassays. A positive RT-PCR test has been used previously to indicate previous  
413 (COVID-19) SARS-CoV-2 infection, though this approach is limited by a high rate of  
414 false negatives and positives in RT-PCR testing, failure in some cases to develop IgG  
415 antibodies (sero-silence or lack of antibody against the same antigenic component of  
416 the virus as the immunoassay uses as a capture antigen) and the lack of RT-PCR  
417 testing availability early in the pandemic (3,5,24). SARS-CoV-2 IgG antibodies were  
418 undetectable in 14 of 267 (5.2%) of previously RT-PCR SARS-CoV-2 viral RNA

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3 419 positive participants in this study. It is unclear if this is due to insufficient/absent  
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5 420 antibody production in these individuals at the time the sample was taken, or due to a  
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7 421 false positive PCR result which may occur in the UK at a rate between 0.8- 4.0% (6).  
8  
9 422 Self-assessment of symptoms for COVID-19 (disease) is a poor indicator of previous  
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11 423 infection, even amongst healthcare workers (25). Additionally, the kinetics of a SARS-  
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13 424 CoV-2 virus infection contributes to the loss of sensitivity of RT-PCR to detect virus  
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15 425 with time, contributing to false negative RT-PCR test results for individuals who may  
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17 426 be late to present for virus detection tests (5,26).  
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24 428 To assess sensitivity and specificity of the AbC-19 LFIA for its ability to detect SARS-  
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26 429 CoV-2 antibody in a laboratory evaluation, we developed a reference standard for  
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28 430 SARS-CoV-2 antibodies, which does not rely on a single test as reference. A similar  
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30 431 approach was used in a recent seroprevalence study in Iceland, whereby two positive  
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32 432 antibody results were required to determine a participant sample as positive for SARS-  
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34 433 CoV-2 antibody (24). Our evaluation of performance metrics for the UK-RTC AbC-19  
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36 434 LFIA to detect antibodies for SARS-CoV-2 gave 97.58% sensitivity and 99.59%  
37  
38 435 specificity. In an evaluation of the AbC-19 tests, Mulchandani *et al.* observed a  
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40 436 specificity of 97.9% (97.2%-98.4%) on a cohort of pre-pandemic samples and report  
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42 437 a sensitivity of 92.5% (88.8% to 95.1%) for detecting previous infections (based on a  
43  
44 438 previous RT-PCR result) or 84.7% (80.6% to 88.1%) against the Roche Elecsys  
45  
46 439 antibody test, which detects IgM/IgG/IgA SARS-CoV-2 antibodies to the nucleocapsid  
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48 440 portion of SARS-CoV-2 (25). In RT-PCR positive individuals from our cohorts, the  
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50 441 AbC-19 test showed a similar sensitivity (92.07%, 87.76%- 95.23%, Table S3).  
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52 442 However, we demonstrate the drawbacks of this approach given that in 12 of 18 AbC-  
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54 443 19 false negatives, none of the four immunoassays used (EuroImmuno, Roche, Abbott  
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3 444 or AbC-19) detected antibodies, suggesting either a false RT-PCR result, a failure to  
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5 445 produce IgG antibodies or sero-reversion before sample collection in these individuals.  
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7 446 Another recent evaluation of the AbC-19 LFIA by Moshe *et al.* determined a sensitivity  
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9 447 of (100% (98.1-100%)) on laboratory sera, using a composite reference standard of  
10  
11 448 antibody positive by either Spike protein ELISA or hybrid DABA assay and specificity  
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13 449 of 99.8% (98.9-100%) against pre-pandemic samples. However, when AbC-19  
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15 450 performance was analysed on matched finger-prick and serum samples against the  
16  
17 451 same antibody standard, a lower sensitivity was observed (finger-prick 69% (53.8-  
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19 452 81.3%), serum 92% (80-97.7%)) (27).  
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26 454 In our study, strong correlation was observed in quantitative score between results on  
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28 455 all immunoassays with the highest observed between EuroImmun and AbC-19 LFIA  
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30 456 (Figure S8, S9). This is to be expected, given both the AbC-19 LFIA and EuroImmun  
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32 457 ELISA detect IgG antibodies against spike protein. Importantly, for the assessment of  
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34 458 immunity to prior natural infection as well as to immunisation, IgG antibodies against  
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36 459 SARS-CoV-2 spike protein detected by laboratory-based EuroImmun ELISA and AbC-  
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38 460 19 LFIA are known to correlate with neutralizing antibodies, which may confer future  
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40 461 immunity (23,28,29). Previous evaluations of sensitivity and specificity reported by  
41  
42 462 Public Health England (PHE), showed a EuroImmun sensitivity of 72% and specificity  
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44 463 of 99%, Abbott with sensitivity of 92.7% and specificity of 100% and Roche with  
45  
46 464 sensitivity of 83.9% and specificity of 100% (30–32). The PHE analyses for each of  
47  
48 465 these tests used previous infection (RT-PCR positive status) as a reference standard,  
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50 466 the limitations of which are discussed above.  
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3 468 In the use of characterised 'known positive' and 'known negative' cohorts, one  
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5 469 limitation of this study is its potential for spectrum bias, whereby our positive-by-two  
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7 470 reference system may artificially raise the threshold for positive sample inclusion,  
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9 471 possibly resulting in the overestimation of the sensitivity of any test evaluated (33).  
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11 472 However, similar issues have been raised when using previous RT-PCR result or  
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13 473 definitive COVID-19 symptoms as inclusion criteria given these will likely skew a  
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15 474 cohort towards more severe disease, especially given issues of RT-PCR availability  
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17 475 outside of hospital settings during the first wave (5). Importantly, our mixed origin of  
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19 476 samples forming the cohort provides a positive cohort for assessing assay sensitivity  
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21 477 that includes individuals from the general public, healthcare workers and from  
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23 478 convalescent plasma programmes. In the absence of a clear gold standard test, our  
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25 479 system relies on no single test (each with their individual shortcomings) and instead  
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27 480 takes an average of three. Our analysis of specificity on only pre-pandemic individuals  
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29 481 (n=223) shows similar specificity (99.55%) to the larger mixed 'known negative cohort'  
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31 482 (n=488, sensitivity 99.59%). We also demonstrate a high level of analytical specificity  
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33 483 of the AbC-19 test with no cross-reactivity against a panel of other respiratory viruses,  
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35 484 including SARS-CoV-1 NL63 and 229E (Table S4).  
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486 Our assessment of the AbC-19 LFIA in a laboratory setting, using characterised  
487 cohorts of known SARS-CoV-2 antibody positive and antibody negative plasma,  
488 shows good performance metrics for its ability to detect SARS-CoV-2 IgG antibodies  
489 following natural infection. We note our use of plasma from venous blood samples, as  
490 opposed to a finger prick blood sample as would be used in rapid testing scenarios  
491 (27). Additionally, when the AbC-19 LFIA was used on our cohort, a number of the  
492 positive results scored low (1/10 using the score card under laboratory conditions,

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3 493 Figure 3), with a faint test band visible to a trained laboratory scientist but perhaps  
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5 494 difficult to identify as positive by individuals performing a single test (Figure S10). This  
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7  
8 495 faint line may be reflective of the longer time from infection for the Northern Ireland  
9  
10 496 cohort used. If the AbC-19 LFIA is to be used in clinical settings it is important to  
11  
12 497 determine if all users observe the same results as observed in this laboratory  
13  
14  
15 498 evaluation.  
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17 499  
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19 500 This assessment of the AbC-19 LFIA does not provide data on how this test will  
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21 501 perform in a seroprevalence screening scenario, but instead provides metrics for the  
22  
23 502 performance of the test, where presence of SARS-CoV-2 antibodies is of interest, as  
24  
25 503 opposed to previous COVID-19 infection. An important potential use of the AbC-19  
26  
27 504 LFIA would be in monitoring the immune response to vaccination, with most vaccines  
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29 505 utilising SARS-CoV-2 Spike protein antigens (34).  
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## 35 507 **Conclusion**

37 508 We present a comprehensive analysis of pre-pandemic and two large pandemic  
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39 509 cohorts (more than 700 individuals) and in a longitudinal analysis showing that IgG  
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41 510 antibodies to SARS-CoV-2 antigens are detectable more than 10 months from positive  
42  
43 511 RT-PCR test. We use antibody positive status as an alternative to RT-PCR positive  
44  
45 512 status as a standard for assessing SARS-CoV-2 antibody assays and show strong  
46  
47 513 performance for the UK-RTC AbC-19 LFIA rapid point of care test in detecting SARS-  
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49 514 CoV-2 antibodies. User experience in future studies in the real world is important and  
50  
51 515 may alter the performance characteristics. Also, the effect of operator training will have  
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53 516 direct effects upon test performance. We welcome further clinical evaluation of the  
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55 517 AbC-19 LFIA in large cohorts of symptomatic and asymptomatic individuals alongside  
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3 518 large studies assessing vaccination outcomes in individuals to fully validate its  
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5 519 implementation across all intended use cases.  
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10 521 **Declarations**

11  
12 522 **Ethics approval and consent to participate**

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14 523 All study participants provided informed consent. This study was approved by Ulster  
15  
16 524 University Institutional Ethics committee (REC/20/0043), South Birmingham REC (The  
17  
18 525 PANDEMIC Study IRAS Project ID: 286041Ref 20/WM/0184) and adhered to the  
19  
20 526 Declaration of Helsinki and Good Clinical Practice.  
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23  
24 527 **Patient and Public Involvement**

25  
26 528 Patients or the public were not involved in the design, or conduct, or reporting, or  
27  
28 529 dissemination plans of our research.  
29

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31 530 **Consent for publication**

32  
33 531 Not applicable.  
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35  
36 532 **Dissemination to participants and related patient and public communities.**

37  
38 533 Links to this work will be included on the study website  
39  
40 534 (<https://www.ulster.ac.uk/coronavirus/research/research-output/pandemic-study>) and  
41  
42 535 participants will be alerted that the work has been published.  
43

44  
45 536 **Data sharing**

46  
47 537 Data are available on reasonable request to the corresponding author.  
48

49  
50 538 **Competing interests:**

51  
52 539 At the time of this study TM and JML acted as advisors to CIGA HealthCare, an  
53  
54 540 industrial partner in the UK Rapid Test Consortium. No personal financial reward or  
55  
56 541 remuneration was received for this advisory role. At the time of submission of this  
57  
58 542 manuscript TM and JML no longer held these advisory positions.  
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60



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3 543 All other authors have no potential conflict of interest to report.  
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13

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15  
16 549 manuscript and associated data within this paper has only been used to build  
17  
18 550 confidence into the overall device design and performance assessment of the UK RTC  
19  
20 551 AbC-19 devices and such work was never commissioned for any government  
21  
22 552 contractual consideration.  
23

24  
25  
26 553 **Authors' contributions:**  
27

28 554 TM, JML conceived the study. LR, JM and TM performed all laboratory analyses. LR,  
29  
30 555 SM and KYN analysed data, KB performed all statistical analyses/interpretations and  
31  
32 556 produced figures. NQ, FJ, GW and PS performed all Roche analyses and provided  
33  
34 557 SHSCT cohort samples. MC, KM and SR performed all Abbott analyses and provided  
35  
36 558 Blood Transfusion cohort samples. TM, RP and AN coordinated participant  
37  
38 559 recruitment, consent and sampling. WB and JML developed online consent forms,  
39  
40 560 questionnaires and databases. LR, JM, AK, AA, GEW, DH, SS, CCS performed  
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42 561 sample collection and processing. LR and TM wrote the manuscript, with significant  
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3 **715 Table 1: Antibody level ratios for assays over time**

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5 **716** Antibody level ratios for assays over time show varying peak levels depending on test.

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7 **717** Calculated by first establishing the median per time period, then calculating log<sub>2</sub> ratio

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9 **718** 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+
<b>EuroImmun</b>	-2.65	1.33	0.2	0.95	1.32	0.47	0.04	-2.01	-2.26	-2.01
<b>Roche</b>	-3.64	3.16	3.05	5.20	5.45	4.14	4.42	-3.54	-3.69	-3.61
<b>Abbott</b>	-5.54	1.64	-0.51	1.12	0.86	0.08	-0.59	-5.13	-5.13	-6.13
<b>Sample number (n=)</b>	223	20	10	52	90	202	53	11	12	11

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26 **719**

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28 **720 Table 2: UK-RTC AbC-19 LFIA performance metrics against known antibody**

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30 **721** positive and known antibody negative cohorts.

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

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3 723 **Figure Legends**  
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8 725 **Figure 1: Two-way correlation scatter plots comparing a) EuroImmun b) Abbott**  
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10 726 **and c) Roche immunoassays.** Pearson  $\chi^2$  test was used to assess correlations. The  
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12 727 results for each test were log transformed to ensure results follow a normal distribution.  
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14 728 Negative agreement shown as blue dots, red dots show positive agreement for the  
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16 729 two immunoassays, whilst black dots show disagreement and grey dots as the  
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18 730 EuroImmun borderline results. Vertical lines mark the Abbott test range 0.25-1.4.  
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20 731 n=880. The graphs show positive correlations between all immunoassays evaluated,  
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22 732 with the fewest disagreement of results between the Log of Roche and the Log of  
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24 733 EuroImmun. Fit lines LOESS, with 95% confidence interval shaded.  
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31 735 **Figure 2: SARS-CoV-2 antibody levels by (a) EuroImmun, (b) Roche, and (c)**  
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33 736 **Abbott, relative to weeks since first reported symptoms or positive PCR result**  
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35 737 **(where data available, n=685).** RT-PCR positive individuals are denoted by red dots,  
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37 738 while individuals with time since symptom data are denoted in black. Dashed lines  
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39 739 delineate  $\log_e$  equivalent of positivity threshold (EuroImmun 1.1, Roche 1.0, Abbott  
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41 740 1.4) for each test, and the negativity threshold for EuroImmun (0.8; borderline result  
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43 741 between the two lines). Black bars indicate median, within IQR (interquartile range)  
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45 742 boxes for EuroImmun/Roche/Abbott value. Red triangles indicate outliers, based on  
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47 743  $1.5 \times$  IQR (interquartile range).  
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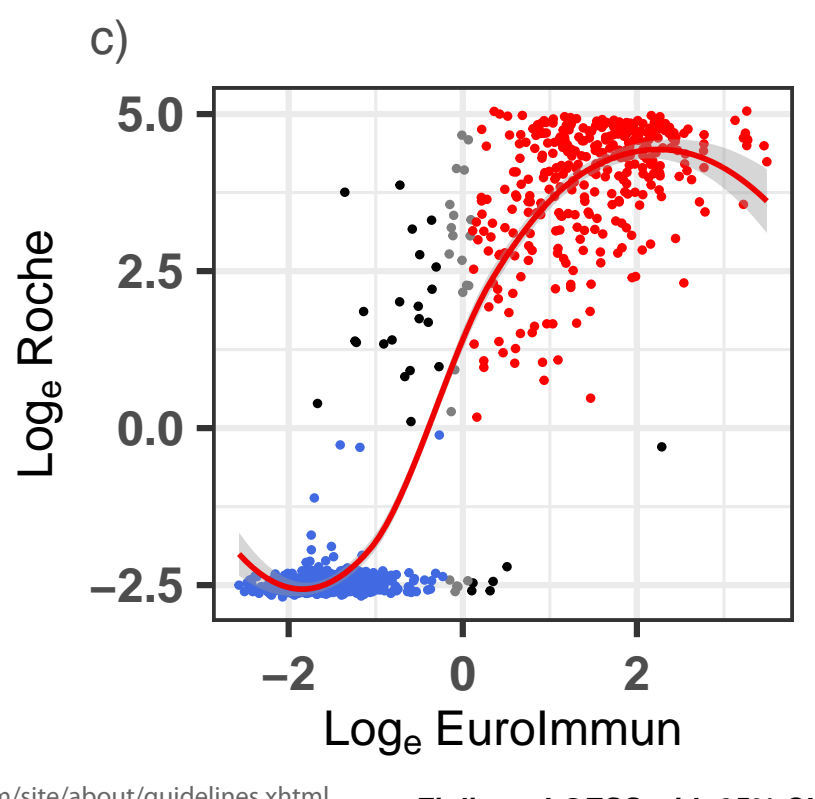
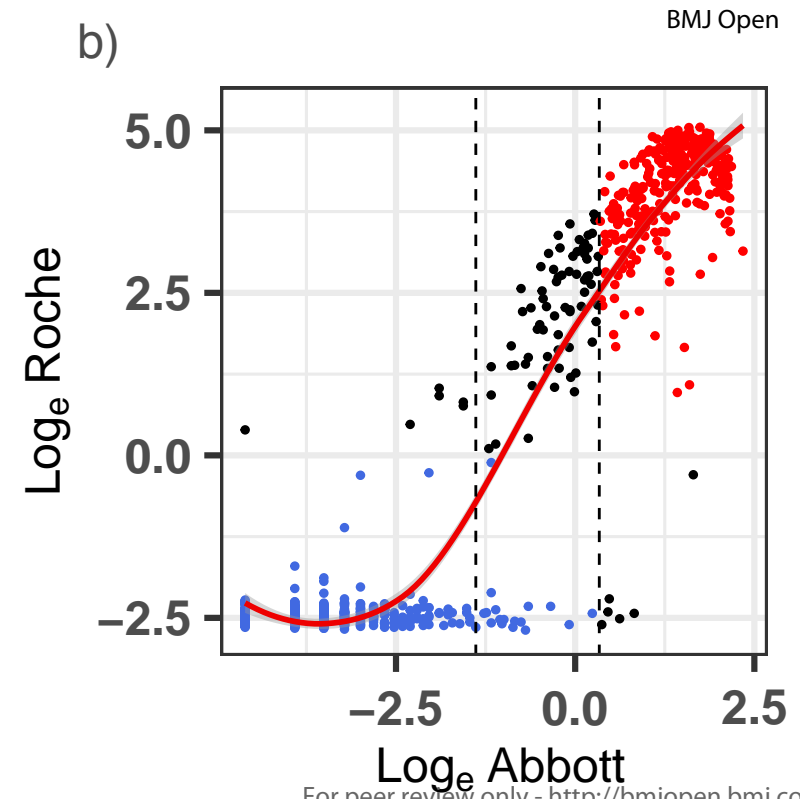
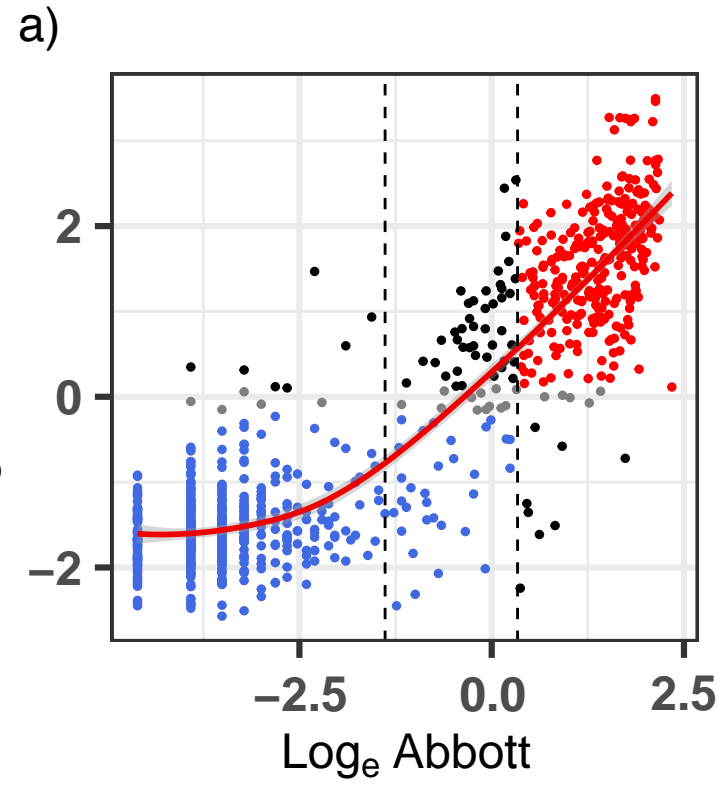
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53 745 **Figure 3: AbC-19 extended cohort (n=818) correlation to a) EuroImmun b) Roche**  
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55 746 **and c) Abbott scores.** Box plots overlaid on scatter plot, comparing AbC-19 TT3 test  
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57 747 scores to EuroImmun, Roche and Abbott quantitative antibody values. Red linear line  
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3 748 of best fit with 95% confidence interval shaded in grey. Black bars indicate median,  
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5 749 within IQR (interquartile range) boxes for EuroImmuno/Roche/Abbott value. Red  
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8 750 triangles indicate outliers, based on  $1.5 \times$  IQR (interquartile range).  
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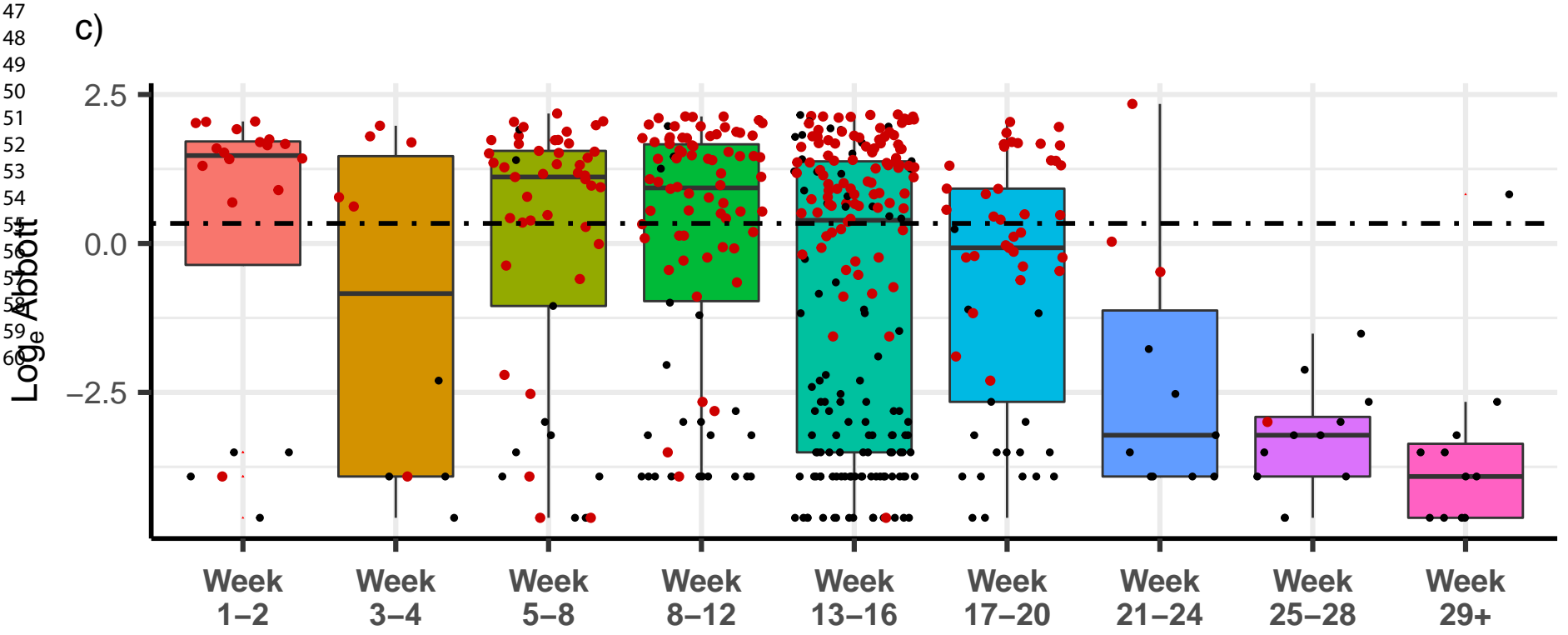
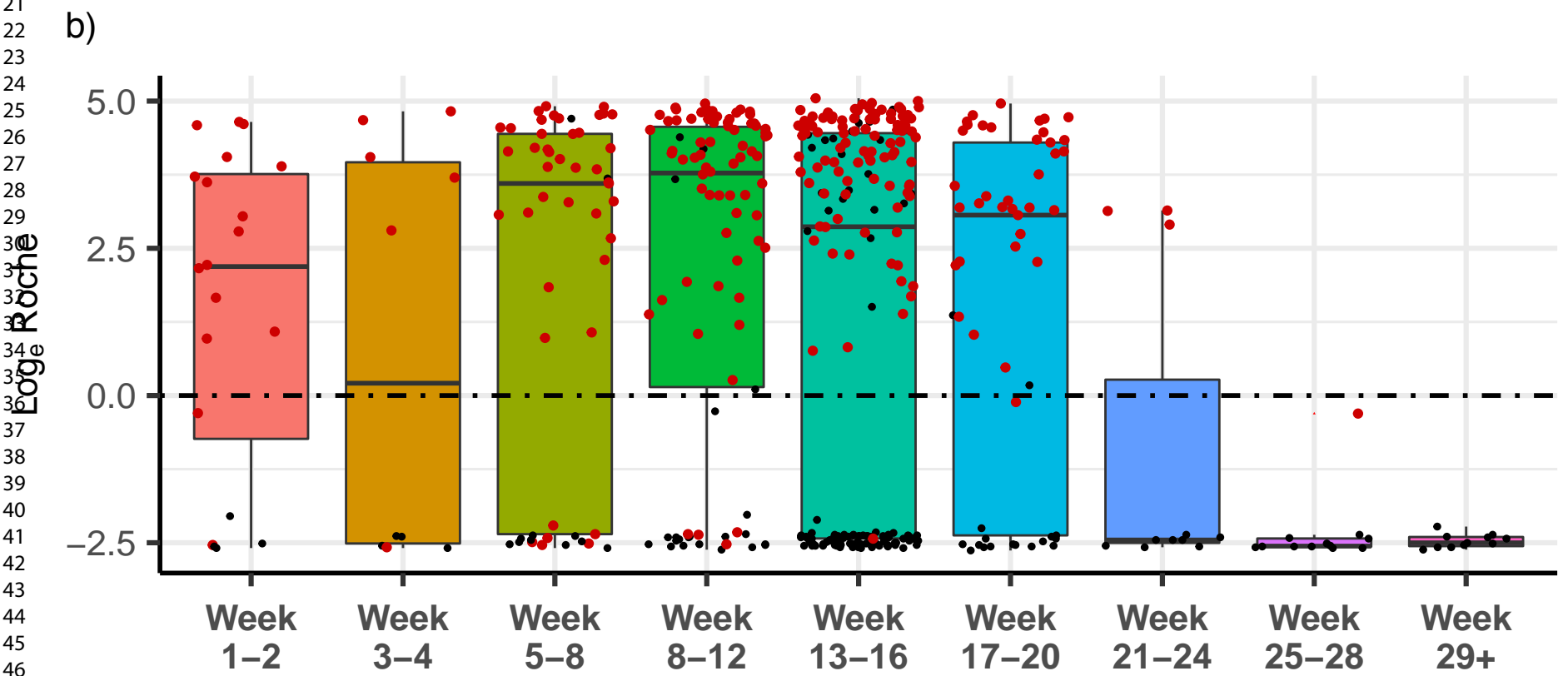
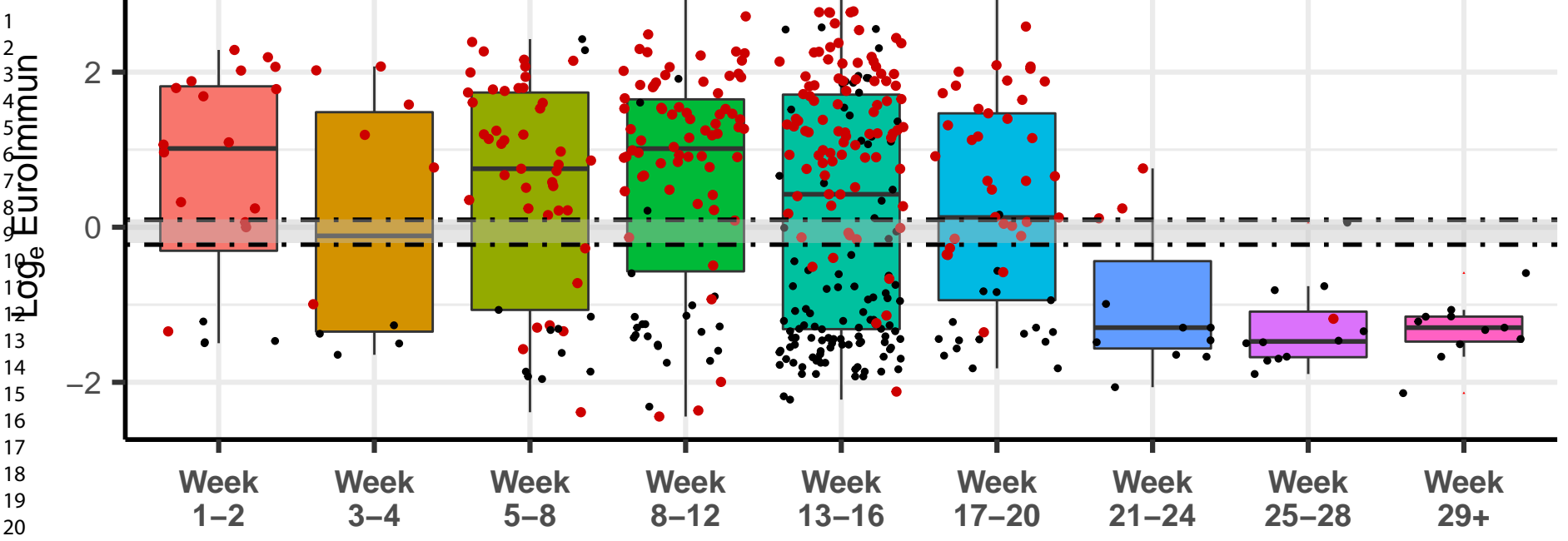
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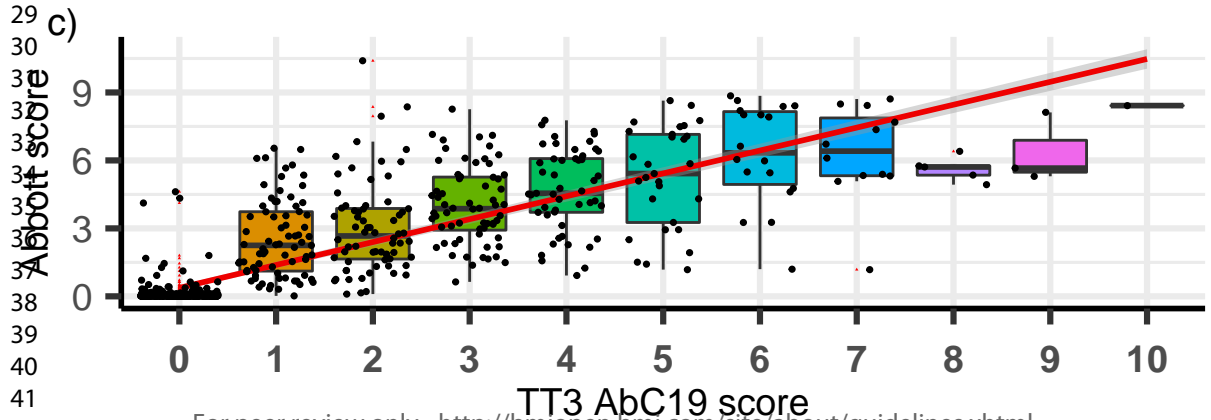
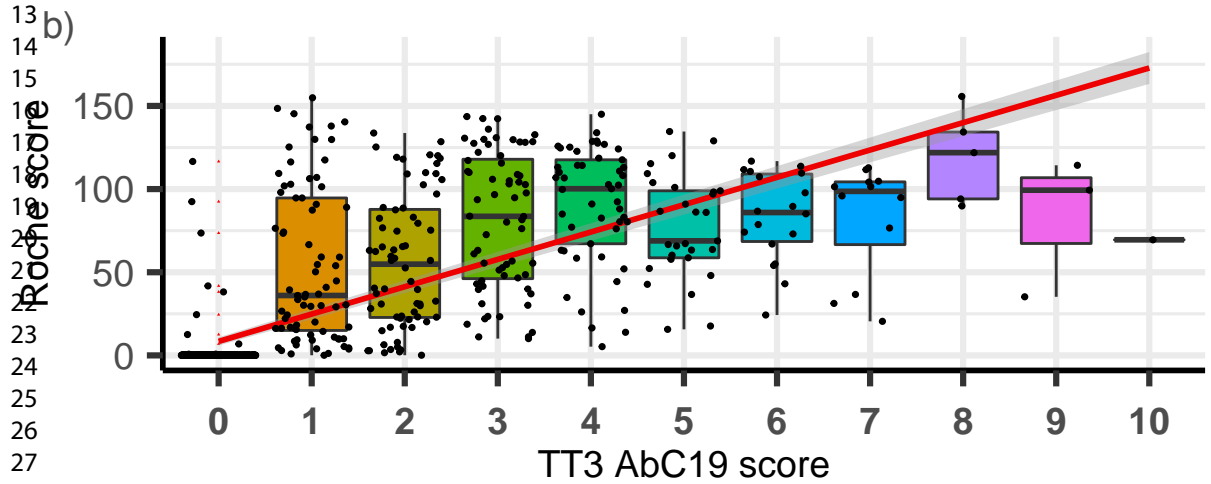
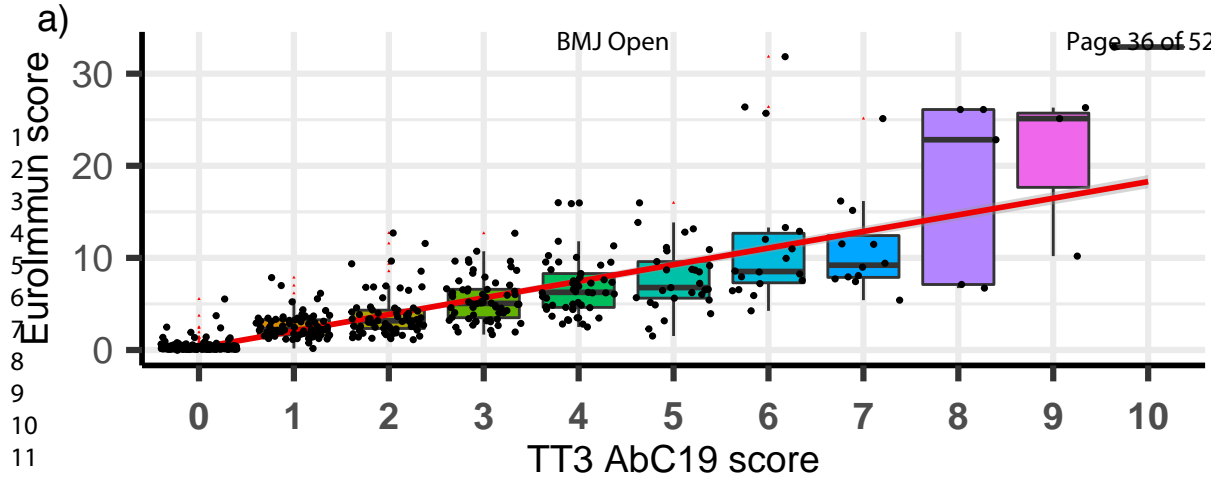


- Category
- Both negative
  - Both positive
  - Disagreement
  - EuroImmun borderline

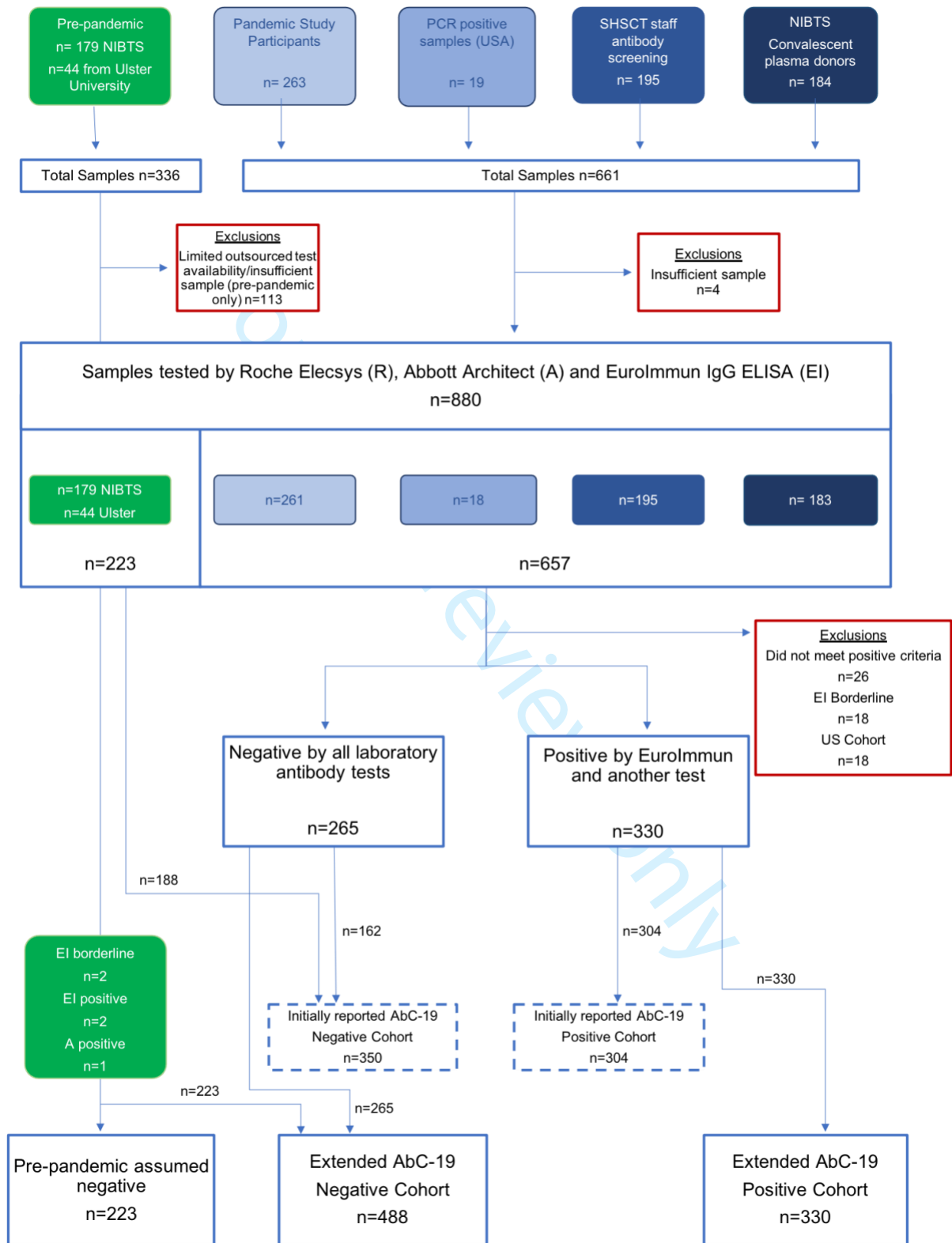
For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

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





Supplementary Materials



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3 **Figure S1: Flow of participant plasma samples through cross-sectional study.**

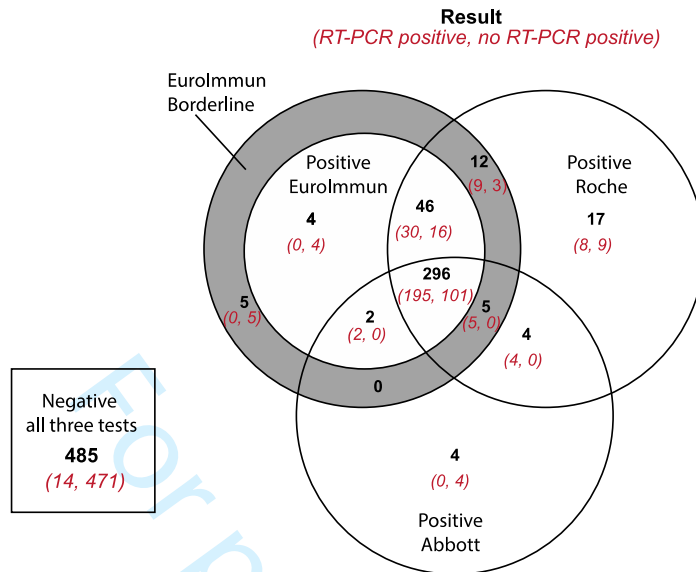
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5 All available samples from participants within each cohort, and the included and  
6 excluded samples at all stages. Freeze thaw cycles were closely monitored for all  
7  
8 excluded samples at all stages. Freeze thaw cycles were closely monitored for all  
9  
10 sample aliquots. Pre-pandemic samples taken forward for Roche, Abbott and  
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12 EuroImmun testing were selected based on aliquot volume and availability.  
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45 **Figure S2: Visual Score card for quantitative interpretation of AbC-19 LFIA test**  
46 **bands.** A scale of 0 (not pictured, negative-no test line visible) to 10 (positive-  
47 strongest test line). Any LFIA scoring 1 or above was classified as positive.  
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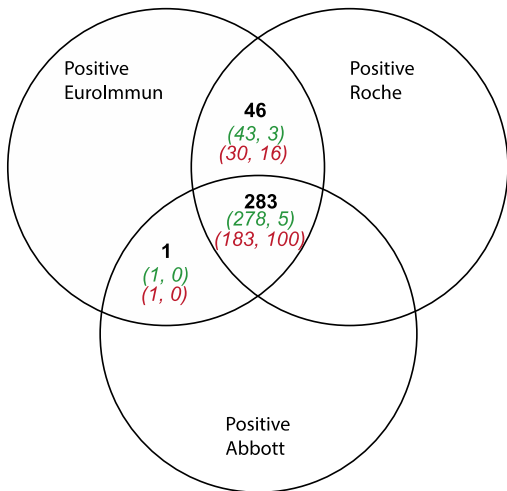
a)

### Laboratory immunoassays (n=880)



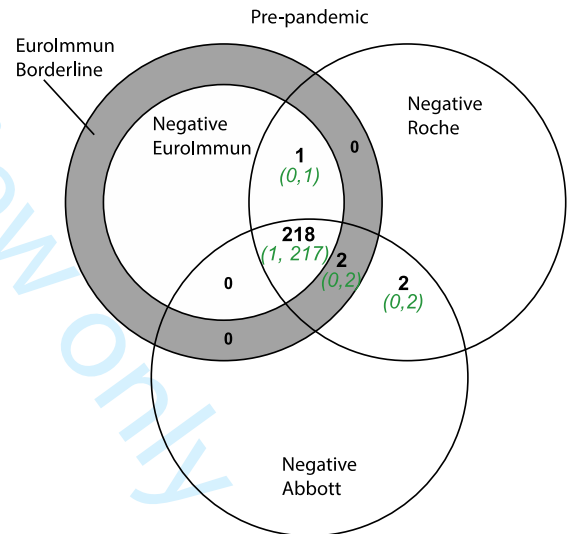
### b) Positive cohort (n=330)

**Result**  
*(AbC-19 positive, AbC-19 negative)*  
*(RT-PCR positive, no RT-PCR positive)*

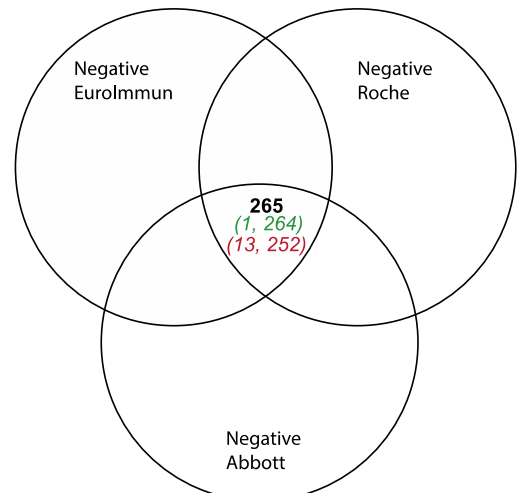


### c) Negative cohort (n=488)

**Result**  
*(AbC-19 positive, AbC-19 negative)*  
*(RT-PCR positive, no RT-PCR positive)*



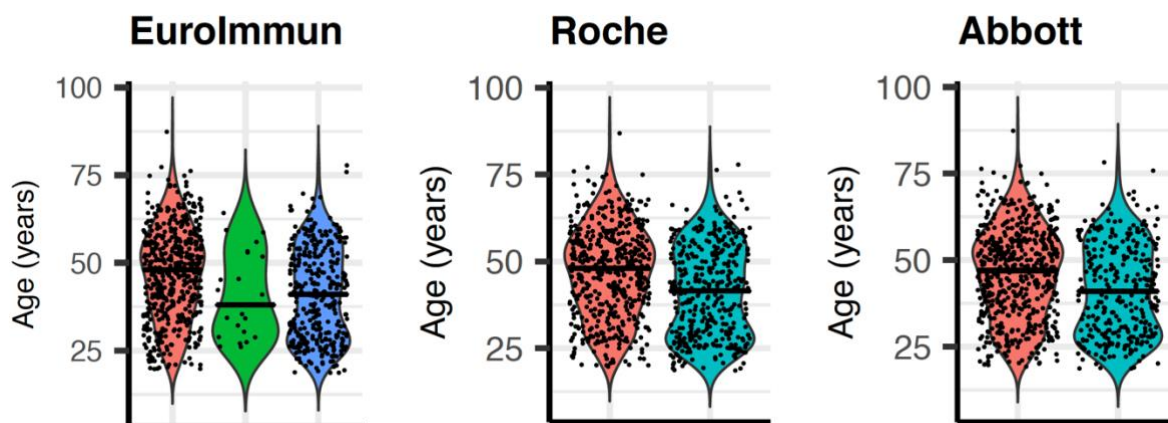
Collected 2020



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3 **Figure S3: Venn diagrams demonstrating result overlap between laboratory**  
4 **assays in a) the initial immunoassay cohort (n=880), b) the positive and c)**  
5 **negative cohorts assessed with AbC-19 TT3.** Result in each circle overlap in bold,  
6 (RT-PCR positive, no RT-PCR positive) denoted in red in brackets below. Where AbC-  
7 19 was analysed, (AbC-19 positive, AbC-19 negative) denoted in green.  
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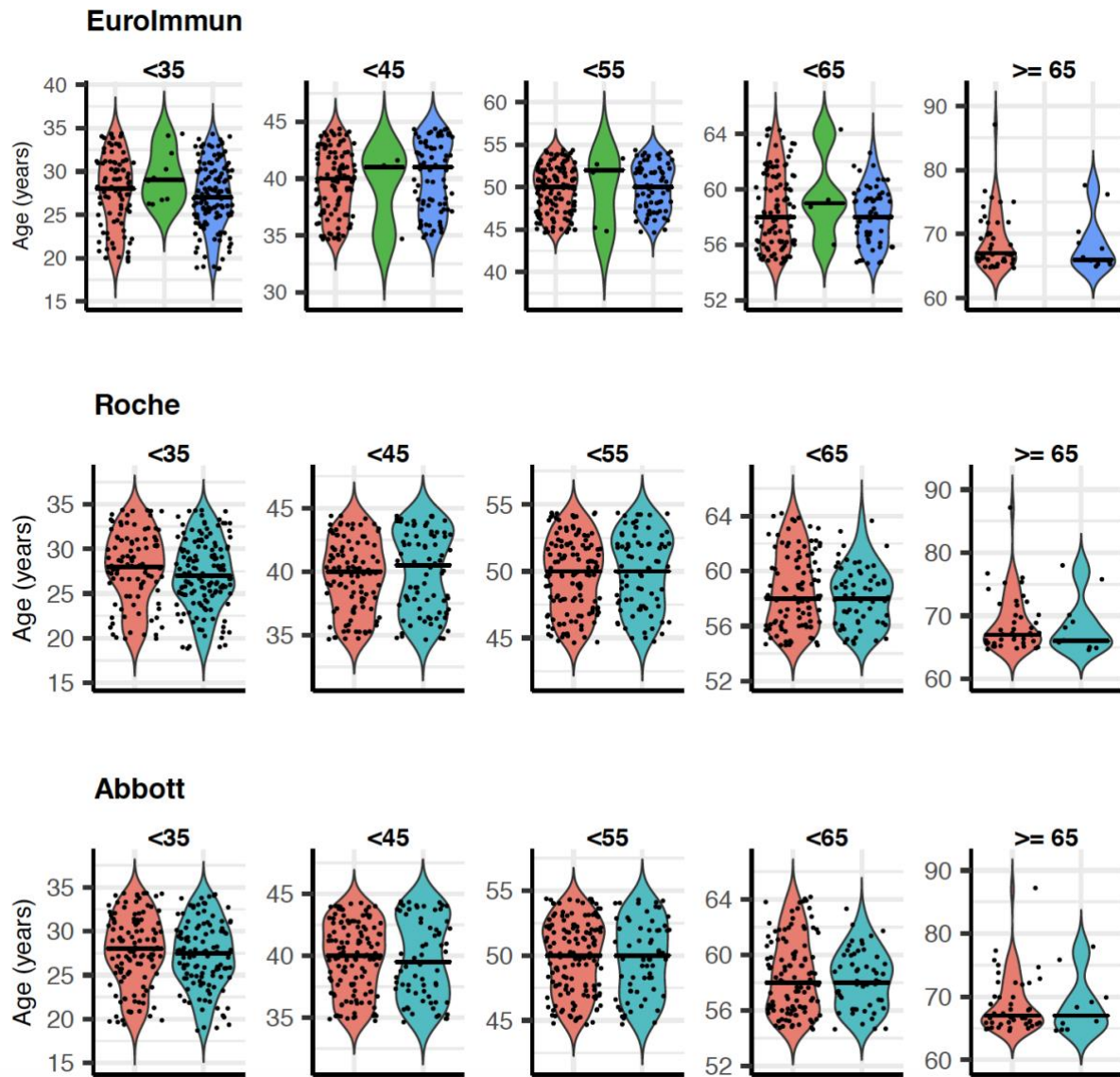
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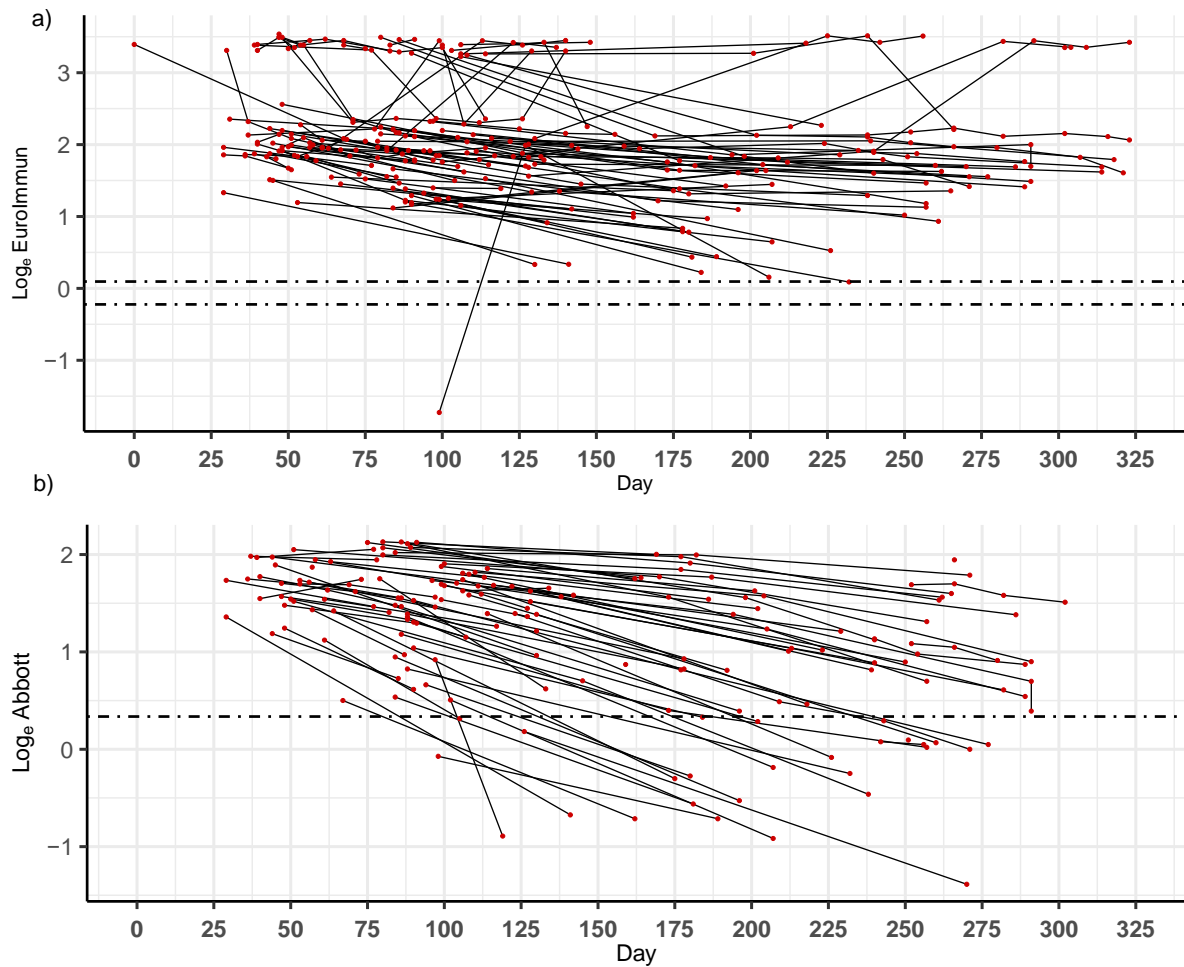
**Figure S4: Age violin plots overlaid with scatter for samples included in correlation analysis (where age data available) n=880.**

The above graphs allow comparison of the distributions and probability density of ages for EuroImmune, 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.

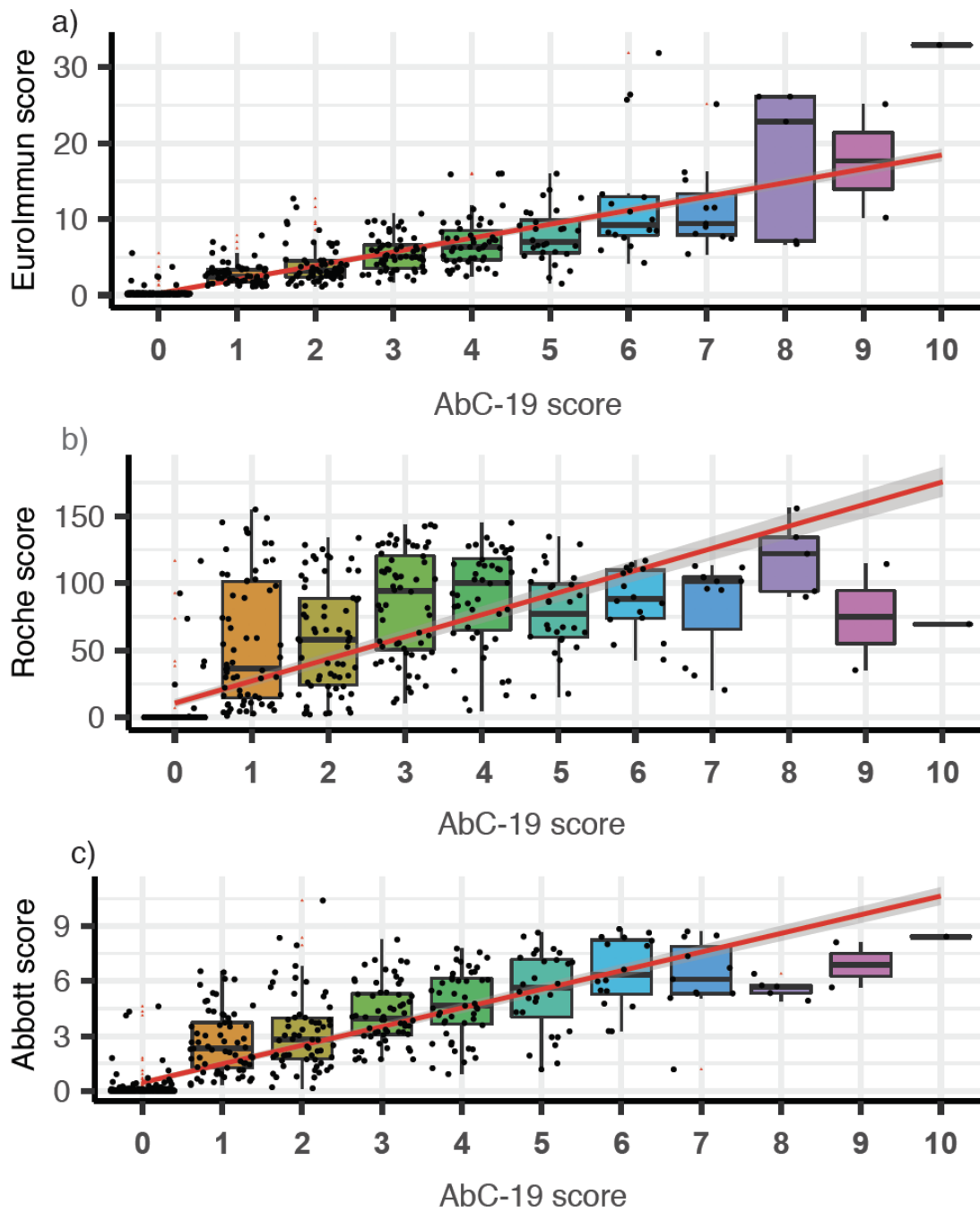


**Figure S5: Age violin plots separated into age groups (where age data available) for samples included in correlation analysis.**

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  $\geq 65$  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).

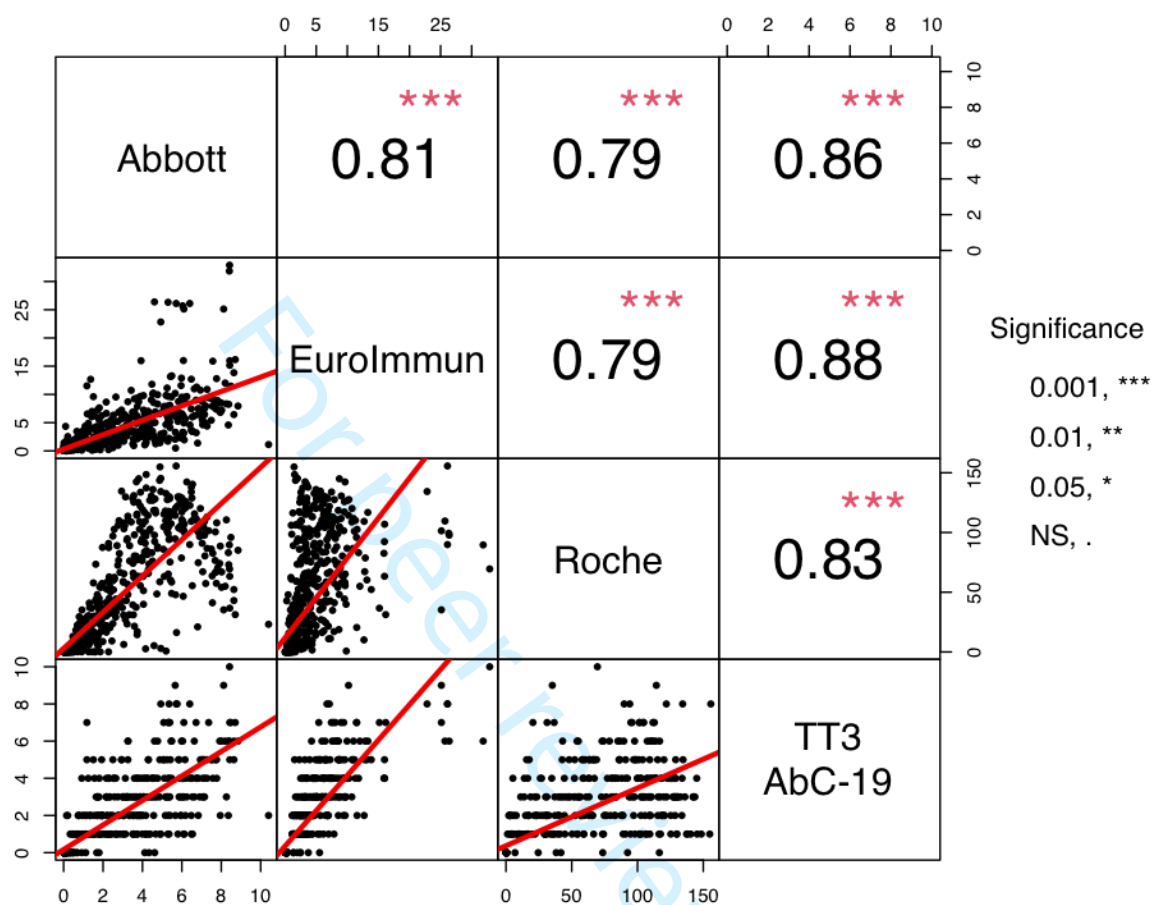


**Figure S6: Longitudinal analysis of convalescent plasma donor sequential samples (2-9 samples per individual) by a) EuroImmun ELISA or b) Abbott immunoassay.** a) n=101 individuals, grey shading indicates borderline region, upper dotted line indicates positivity threshold (1.1), lower dotted line indicates 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.

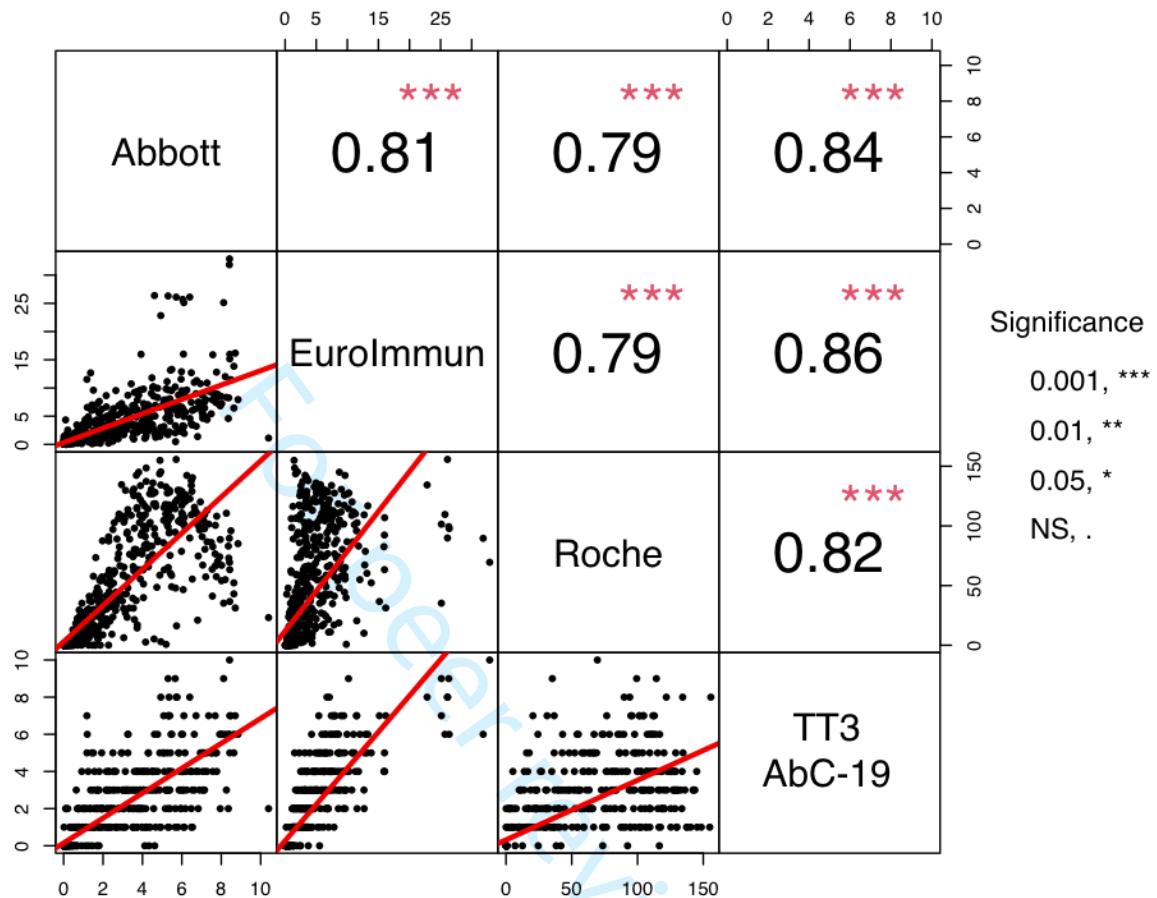


Linear fit line with 95% CI

**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  $1.5 \times$  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
<b>EuroImmun ELISA</b>	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
<b>Roche Elecsys immunoassay</b>	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
<b>Abbott Architect SARS-CoV-2</b>	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
<b>3 AbC-19</b>	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%)
Eurolmmun	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.

EuroImmune Anti-SARS-CoV-2 ELISA-IgG (EuroImmune, 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 EuroImmune calibrator, with a score of < 0.8 determined negative,  $\geq 0.8$  to <1.1 borderline and  $\geq 1.1$  positive. For samples provided by NIBTS, EuroImmune 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  $\geq 1.0$  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

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3 20/B764-01). Results are reported by dividing the sample result by the calibrator result.  
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5 The result unit for the SARS-CoV-2 IgG assay is Index (Sample/Calibrator). A ratio of  
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7 < 1.4 is determined negative and  $\geq 1.4$  is determined positive.  
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### 10 11 12 *Analytical specificity and sensitivity assessment* 13

14 Four virology samples (H5N1 influenza serology 7/150, RSV serology 16/284,  
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16 Influenza B 9/222 and Bordetella Pertusis 89/530) were obtained from NIBSC  
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18 (National Institute for Biological Standards, Herts, UK). An additional 30 serology  
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20 samples from known virus infections were a kind gift from Sugentech, Seoul, Korea.  
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22 15 of these virology samples were obtained from Trina (Trina Bioreactives AG,  
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24 Switzerland) from 5 different individuals per virus (Influenza A IgG, Influenza B IgG  
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26 and RSV IgG). A further 15 of these virology samples were obtained from AbBaltris,  
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28 Kent from 5 different individuals per virus (Haemophilus Influenza IgG, Seasonal  
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30 Coronavirus NL63 and 229E Seasonal Coronavirus). All these serology samples  
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32 alongside a panel of 6 external standard research reagents (Table S4; NIBSC; Cat:  
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34 20/118 and 20/130) were assessed on the TT3 AbC-19 LFIA to confirm analytical  
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36 specificity and sensitivity.  
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Section & Topic	No	Item	Reported on page #
<b>TITLE OR ABSTRACT</b>			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
<b>ABSTRACT</b>			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
<b>INTRODUCTION</b>			
	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
<b>METHODS</b>			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
<i>Participants</i>	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
<i>Test methods</i>	9	Whether participants formed a consecutive, random or convenience series	6
	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
<i>Analysis</i>	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
<b>RESULTS</b>			
<i>Participants</i>	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
<i>Test results</i>	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
<b>DISCUSSION</b>			
	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
<b>OTHER INFORMATION</b>			
	28	Registration number and name of registry	N/a
	29	Where the full study protocol can be accessed	Ethics approval documents

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