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# Evaluation of 2 commercial anti-SARS-CoV-2 antibody assays in an immunocompetent and immunocompromised inpatient population with COVID-19

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## ABSTRACT

**Introduction:** During the COVID-19 pandemic, widespread introduction of SARS-CoV-2 antibody testing was introduced without a full understanding of the assays performance or the antibody kinetics following infection with SARS-CoV-2. **Methods:** We performed an evaluation of 2 anti-SARS-CoV-2 antibody assays with a more detailed look into the effect of immune status on antibody sensitivity. **Results:** Both assays demonstrated 100% specificity. The overall sensitivity of the Roche was 92.1% at  $\geq 14$  days and 94.8% at  $\geq 21$  days, and the overall sensitivity of the Abbott was 94.4% at  $\geq 14$  days and 98.2% at  $\geq 21$  days. 7/41 (17%) of patients included in this cohort were immunocompromised. Seroconversion was seen less commonly in the immunocompromised (4/7 [57.1%] seroconverted) and after excluding these patients 100% sensitivity was seen in both assays at  $\geq 21$  days. **Discussion:** Performance of both assays in the immunocompetent appeared excellent after 21 days postsymptom onset. Both assays are highly specific.

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## 1. Introduction

As a part the COVID-19 pandemic response there has been an unprecedented introduction of serological testing. The assays predominantly used in the UK are the Roche Elecsys anti-SARS-CoV-2 antibody assay and the Abbott SARS-CoV-2 IgG assay. The Roche Elecsys anti-SARS-CoV-2 combined IgM-IgG assay is a modified double sandwich electrochemiluminescence immunoassay (ECLIA) which detects anti-SARS-CoV-2 IgM and IgG targeted against the SARS-CoV-2 virus nucleocapsid (N). The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) which detects anti-SARS-CoV-2 IgG targeted against the SARS-CoV-2 virus nucleocapsid (N).

A rapid evaluation of the Roche assay performed between the 5th and 7th May 2020 by Public Health England (PHE) using days from symptom onset rather than days from PCR confirmation used samples from 93 SARS-CoV-2 convalescent patients and 472 negative samples and found a specificity of 100%, and a sensitivity of 83.9% ((PHE) PHE). A European diagnostic lab evaluation of similar size found reported a sensitivity of 83.5% for this assay (Naaber et al., 2020). A more in depth PHE evaluation of the Roche assay with a longer period of follow-up reported a 97.2% sensitivity at  $\geq 20$  days using

a set of 536 samples ((PHE) PHE). Antibody responses were sustained up to 73 days postsymptom onset and up to 82 days post a positive PCR result ((PHE) PHE). An evaluation from the United States reported 100% sensitivity after 18 days postsymptom onset (Manthei et al., 2021).

A rapid evaluation of the Abbott assay by PHE using 122 samples from 31 patients reported lower sensitivity of 92.7%  $\geq 14$  days postsymptom onset and 93.5%  $\geq 21$  days postsymptom onset, with a lower specificity of 93.9% ((PHE) PHE). In the larger PHE evaluation of 536 positive samples and 994 prepandemic samples, a sensitivity of 92.7% was reported at  $\geq 20$  days postsymptom onset, and specificity of 99.9% reported for the Abbott assay ((PHE) PHE). An evaluation from a US diagnostic laboratory reported 100% sensitivity after day 17 postsymptom onset (Bryan et al., 2020).

Both the Roche and the Abbott assays failed to meet UK Medicines and Healthcare products Regulatory Agency (MHRA) Target Product Profile (TPP) for “enzyme immunoassays” for SARS-CoV-2, which states the assays should have a sensitivity greater than 98% with 95% confidence intervals of 96% to 100% on specimens collected  $>20$  days when tested on a group of at least 200 positive cases (Target product profile - antibody tests to help determine if people have immunity to SARS-CoV-2, 2020). With “optimization of assay thresholds” the Roche assay met the MHRA standard for sensitivity, although the Abbott did not.

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Conversely it has been reported that up to 8.5% of those with confirmed SARS-CoV-2 infection do not seroconvert at all, and that this is more common in those with mild or asymptomatic infection (Staines et al.). It is now also reported that IgG responses to SARS-CoV-2 can wane quickly and seroreversion can be seen (Ibarrondo et al., 2020, Liu et al., 2020, Seow J et al., 2020).

Published data on the antibody response in the immunocompromised are sparse and largely confined to individual case reports and case series and one small study of immune responses in renal transplant patients (Babel et al., 2020; Hartzell et al., 2020; Lucchini et al.; Meca-Lallana et al., 2020; Thornton, 2020; Wang et al., 2020; Wei et al., 2020; Woo et al., 2020a; Xia et al., 2020). Data on performance of these assays in severe vs. nonsevere groups are limited.

Here we present the results of an evaluation exercise of these 2 assays including a more detailed look at differences in sensitivity, time to seroconversion, and antibody waning in immunocompetent and immunocompromised groups.

## 2. Methods

For the uncertainty calculation an in-house internal quality control (IQC) was prepared using a patient sample and serially diluted. For the specificity calculation, 50 pre-pandemic samples collected between July and September 2018 from 50 separate patients were retrieved as “negative” samples. For the sensitivity calculations a larger sample set was identified by cross-matching a list of current inpatients in our hospital trust who were admitted 7 to 14 days previously against a list of all confirmed COVID-19 patients that have been seen by our trust during the pandemic (all patients testing positive for SARS-CoV-2 RNA using a PCR test). Surplus serum samples were retrieved from the virology archive for retrospective SARS-CoV-2 serological testing, and prospective SARS-CoV-2 antibody testing was also undertaken on surplus serum obtained from Blood Sciences laboratory once routine biochemistry testing was completed until each patient was discharged or the period of sample gathering ended. Sample collection and storage occurred between March and May 2020. Clinical information on age, ethnicity, presence of absence of immunocompromise, and ITU admission was collected.

Patient serum samples were tested using the Roche Elecsys anti-SARS-CoV-2 antibody assay on a Cobas e801 analyzer and using the Abbott SARS-CoV-2 IgG assay on a Abbott Architect analyzer. For the Roche Elecsys anti-SARS-CoV-2 combined IgM-IgG assay Cut-off Index (COI)  $\geq 1.0$  is reported as reactive, and COI  $<1.0$  is reported as nonreactive. For the Abbott SARS-CoV-2 IgG assay, COI  $\geq 1.4$  is reported as reactive, and COI  $<1.4$  is reported as nonreactive.

Measurement of anti-SARS-CoV-2 antibody was performed following the manufacturer's instructions with both assays. Measured COIs were measured and documented. Information on, date of onset of symptoms, and immune status were collected.

## 3. Results

### 3.1. Assay verification

Local verification of both assays was undertaken prior to implementation. This included external quality assessment panels, inter-laboratory sample exchange and replicate testing of an internal quality control. Observed maximal coefficient of variation above the assay cut-off was 2.61% using Roche and 2.32% using Abbott.

### 3.2. Specificity

A panel of 50 pre-pandemic serum samples collected July to September 2018 was tested as negative controls. Negative results were recorded for 50/50 (100%) samples using both the Roche and Abbott assays, suggesting both assays had a 100% specificity.

### 3.3. Patient and sample exclusions for sensitivity calculations

Cross-matching of confirmed Covid-19 cases with a list of all admissions admitted 7 to 14 days previously identified 79 patients. 23 sera with insufficient sample volume for testing (less than 0.2 mL) were excluded. 24 patients (all samples) were excluded due to insufficient time points to assess (minimum 3 time points). A further 14 patients were excluded with unclear symptom onset date.

A total sample set of 388 samples from 41 patients was available for testing. These samples were taken between March and June 2020. Sample collection date postsymptom onset ranged 1 to 120 days. The COIs measured on testing each sample are represented in Fig. 1.

### 3.4. Patient demographics

Patient age ranged from 30 to 96 years (mean 65 years). 23/41 (56.1%) were male, 18/41 (43.9%) were female. 21/46 (45.6%) were admitted to the intensive therapy unit (ITU), 6/46 (13%) died during their admission.

Seven patients were immunocompromised; 1 on rituximab and long-term steroids for rheumatoid arthritis (RA), 1 on rituximab for lymphoma, 1 new diagnosis of leukemia, 1 renal transplant on long-term prednisolone, azathioprine and ciclosporin, 1 uncontrolled HIV,

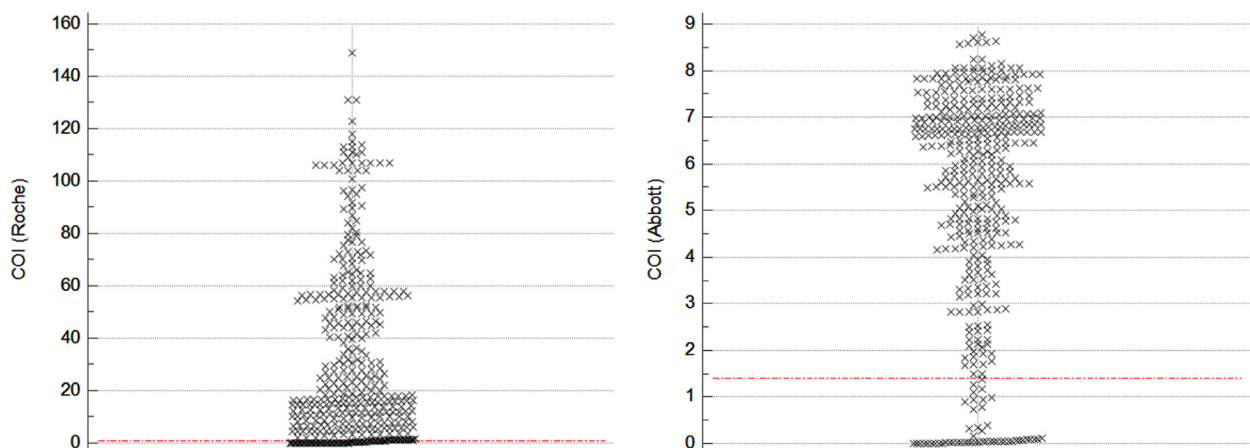


Fig. 1. COI values for all samples tested taken from PCR positive patients (all time points). Red dashed line denotes manufacturer's interpretation threshold. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1 on long-term steroids and hydroxychloroquine for systemic lupus erythematosus (SLE), 1 on long-term mesalazine for Crohns disease.

3.5. Seroconversion

38/41 (92.7%) patients developed detectable anti-SARS-CoV-2 antibody on both assays evaluated. 3/41 (7.3%) had no detectable antibody response with periods of follow-up for these 3 patients being 18, 20, and 27 days postsymptom onset respectively. Subsequent serum samples collected from these patients after our data collection also had negative anti-SARS-CoV-2 IgM/IgG results. These samples were collected at 185, 81, and 50 days postsymptom onset respectively. No additional antibody testing was undertaken in the interim time periods. The severity of illness in the 3 patients who did not seroconvert was moderate, critical, and moderate, respectively.

Only 7 patients had samples available with enough frequency from early illness (at least 1 serum every 72 hours until seroconversion) to detect the approximate time of seroconversion postsymptom onset by measuring a negative and then a positive antibody on serial testing. Using the Roche assay seroconversion was seen by days 6, 8, 10, 11, 12, 14, and 19 respectively, and using the Abbot assay seroconversion was seen by days 6, 8, 10, 12, 12, 14, and 19 days, respectively, in these 7 patients. Mean time to seroconversion was 11 days for both assays assessed.

Patients were observed to seroconvert up to 36 days postsymptom onset. Detectable antibody was observed as early as 4 days postsymptom onset with the Roche, and 2 days postsymptom onset with the Abbott. In these patients, antibody was detected on the initial serum sample, and so it was not clear at what time point seroconversion actually occurred.

3.5.1. Sensitivity by time point

Sensitivity for both assays varied by time point postsymptom onset. In early infection sensitivities were low for both assays. At day 16 to 20 postsymptom onset only 80.4% of samples tested positive

using the Roche assay, and only 82.4% positive using the Abbott. Sensitivity increased to 97.1% for the Roche assay and 94.1% for the Abbott by day 21 to 25 postsymptom onset. Between 31 and 40 days postsymptom onset both assays had 100% sensitivity but after this time point sensitivity again dropped as antibody became undetectable in 1 patient with serum sent late in the course of illness. Sensitivity by time point for both assays is summarized below (Table 1). The overall sensitivity of the Roche was 92.1% at ≥14 days and 94.8% at ≥21 days. The overall sensitivity of the Abbott was 94.4% at ≥14 days and 98.2% at ≥21 days.

3.5.2. Sensitivity in immunocompetent and immunocompromised patients

34/41 (83%) patients were immunocompetent. We found that 100% of immunocompetent patients seroconverted with both assays. Using samples from immunocompetent patients, sensitivity of the Roche assay was 97.9% [95.1%, 99.3%] at ≥14 days, and 100% [97.8%, 100.0%] at ≥21 days. Sensitivity of the Abbott assay was 98.3% [95.7%, 99.5%] at ≥14 days and 100.0% [97.8%, 100.0%] at ≥21 days.

7/41 (17%) of patients in our cohort were immunocompromised. Samples taken from immunocompromised patients had numerically lower rates of positivity at each time point. Only 4/7 (57.1%) of immunocompromised patients seroconverted and only 2 of these had seroconverted by days 21 to 25 postsymptom onset. Seroconversion by time point was similar for both assays tested. A patient with a new diagnosis of leukemia and 2 patients on rituximab did not demonstrate any antibody response in our cohort. Seroconversion did occur in 1 patient with uncontrolled HIV, a patient on mesalazine for Crohns, a patient on long-term prednisolone and azathioprine for SLE and a renal transplant patient.

When analyzing samples taken from immunocompromised patients only, observed sensitivity at ≥14 days was 72.6% for the Roche and 85.7% for the Abbott. Sensitivity at ≥21 days was 81% for the Roche and 95.2% for the Abbott (Table 2).

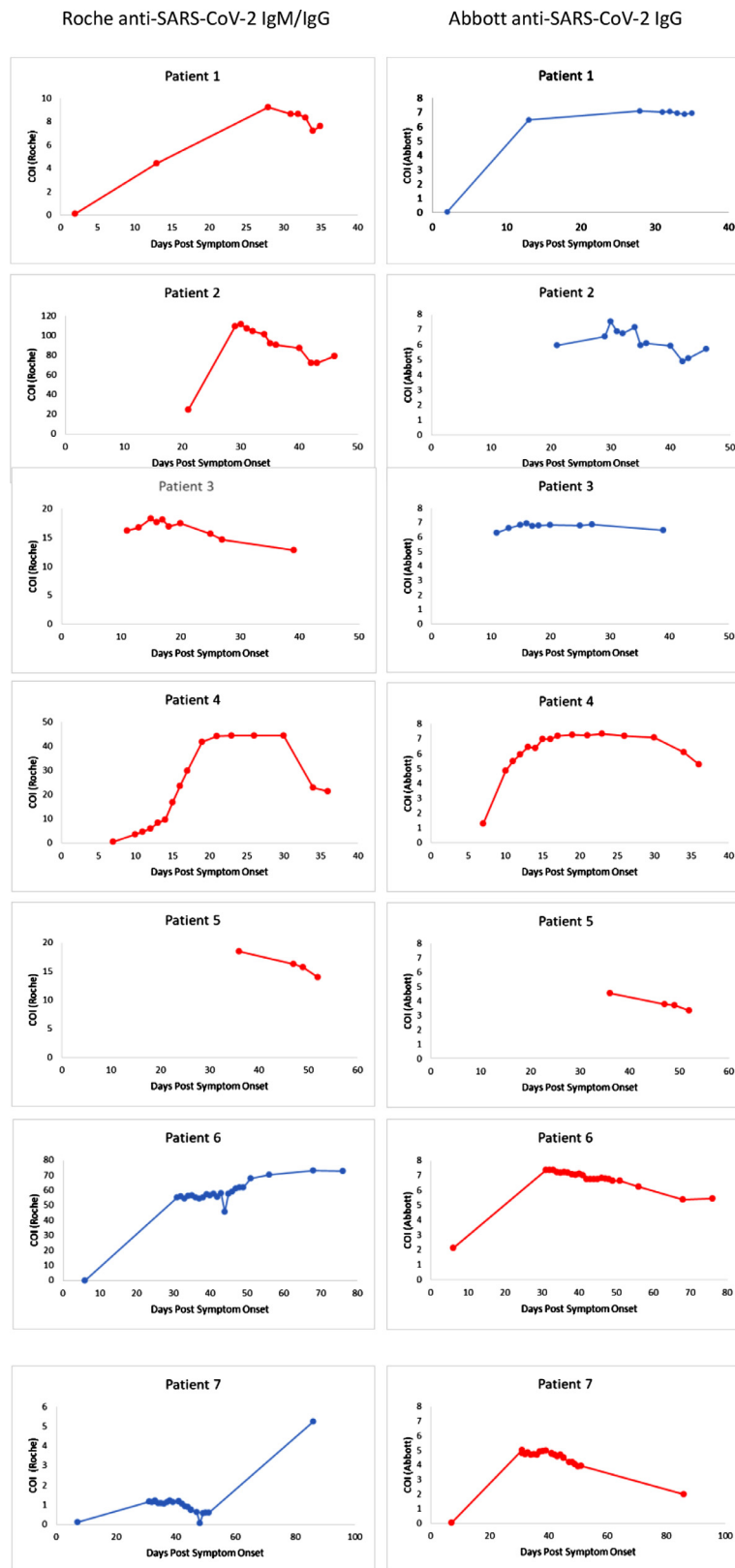
**Table 1**  
Summary of sensitivity by time point.

Days postsymptom onset	Samples (n)	Roche		Abbott	
		Antibody detected	Sensitivity [95% CI]*	Antibody detected	Sensitivity [95% CI]*
1–5	14	5	35.7% [12.8%, 64.9%]	5	35.7% [12.8%, 64.9%]
6–10	36	15	41.7% [25.5%, 59.2%]	19	52.8% [35.5%, 69.6%]
11–15	55	48	87.3% [75.5%, 94.7%]	47	85.5% [73.3%, 93.5%]
16–20	51	41	80.4% [66.9%, 90.2%]	42	82.4% [69.13%, 91.6%]
21–25	34	33	97.1% [84.7%, 99.9%]	32	94.1% [80.3%, 99.3%]
26–30	39	37	94.9% [82.7%, 99.4%]	37	94.9% [82.7%, 99.4%]
31–35	44	44	100.0% [91.7%, 100.0%]	44	100.0% [91.7%, 100.0%]
36–40	30	30	100.0% [88.4%, 100.0%]	30	100.0% [88.4%, 100.0%]
41–45	34	30	88.2% [72.6%, 96.7%]	34	100.0% [89.7%, 100.0%]
46–50	29	25	86.2% [68.3%, 96.1%]	29	100.0% [88.1%, 100.0%]
51–120	22	21	95.5% [77.2%, 99.9%]	22	100.0% [84.6%, 100.0%]
≥14 days	304	280	92.1% [88.5%, 94.9%]	290	95.4% [92.4%, 97.5%]
≥21 days	232	220	94.8% [91.1%, 97.3%]	228	98.2% [95.6%, 99.5%]

\* Confidence intervals were calculated assuming a 10% seroprevalence.

**Table 2**  
Comparison of sensitivities in all patients, immunocompetent vs. immunocompromised, and ITU vs. non-ITU, by time point.

		Number of samples (n)	Roche sensitivity	Abbott sensitivity
All	≥14 days	304	92.1% [88.5%, 94.9%]	95.4% [92.4%, 97.5%]
	≥21 days	232	94.8% [91.1%, 97.3%]	98.2% [95.6%, 99.5%]
Immunocompetent	≥14 days	234	97.9% [95.1%, 99.3%]	98.3% [95.7%, 99.5%]
	≥21 days	169	100.0% [97.8%, 100.0%]	100.0% [97.8%, 100.0%]
Immunocompromised	≥14 days	70	72.9% [60.9%, 82.8%]	85.7% [75.3%, 92.9%]
	≥21 days	63	81.0% [69.1%, 89.8%]	95.2% [86.7%, 99.0%]



**Fig. 2.** Waning of antibody by patient and assay. Left column contains results from the Roche assay, right column contains results from the Abbott assay. Red lines are used when waning is observed, blue lines are used when waning is not observed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Since data collection for this project finished, Abbott introduced the option of a “grayzone” in reporting of results of the Abbott anti-SARS-CoV-2 IgG assay on both ARCHITECT™ and Alinity systems. Laboratories have the option of reporting COIs of 0.5 to <1.4

as a grayzone or of just continuing with reactive/nonreactive result reporting. Reactive results are still reported for  $\text{COI} \geq 1.4$  and only reactive results have been included in these sensitivity analyses.

### 3.5.3. Antibody waning and seroreversion

One of 41 patients (2.4%) subsequently lost their detectable antibody response and reverted to seronegativity. This patient was immunocompromised on ciclosporin, prednisolone and azathioprine following a renal transplant. Serial samples from this patient had undetectable anti-SARS-CoV-2 antibodies by the Roche assay, although a waning antibody response was detected using the Abbott assay. With the latter, weak reactivity was seen initially (peak COI 1.19), and seroreversion occurred by day 43 postsymptom onset. Interestingly a late sample was sent at day 86 and antibody was again detectable at a higher COI of 5.23 (see patient 7 in Fig. 2). No boost in the antibody response was seen using the Abbott assay. The patient had been discharged in the interim and it is not known if there were any further exposure events which may have boosted antibody occurred. A sample mix-up cannot be excluded.

Antibody waning was defined as a progressive sustained average drop in COIs over serial time points. Waning in antibody response was seen in 7 patients—6 using the Roche assay and 4 using the Abbott assay (Fig. 2). All were immunocompetent except the renal transplant patient (patient 7 in Fig. 2) who demonstrated seroreversion.

## 4. Discussion

In this evaluation both the Roche anti-SARS-2 antibody assay and the Abbott anti-SARS-2 IgG assay demonstrated 100% specificity in keeping with high specificities observed in other evaluations (Abbott; Roche; (PHE) PHE; (PHE) PHE; (PHE) PHE). Testing of replicate samples suggested reproducibility was very good and the performance of these assays in our lab was consistent.

Early central evaluations, and some local evaluations have used small sample sizes which may have affected sensitivity calculations ((PHE) PHE; (PHE) PHE; Schnurra et al., 2020). The original rapid PHE evaluations which led to the NHS Improvement (NHSi) recommendations for use of these assays drew criticism due to low sample size, and insufficient information about the patients from whom the samples were taken (Mahase, 2020). Studies into assay performance in the immunocompromised have not been published and data on the antibody response in immunocompromised populations are sparse.

The group of patients from which these samples were gathered were a typical inpatient population. No staff samples or outpatient samples were included. Male to female ratio, and average age and severity were typical for an inpatient population around peak. Testing a large sample size over time points ranging from 1 to 120 days postsymptom onset we found that 7.3% of patients remained antibody negative which is similar to a previous report (Staines et al.).

The sensitivity of both assays when analyzed by time point sensitivity remained low until day 21 postsymptom onset consistent with PHE data ((PHE) PHE). All the immunocompetent patients in our cohort that seroconverted did so on both assays. It was observed that seroconversion occurred less frequently in immunocompromised patients and when samples from immunocompromised patients were excluded, sensitivity of both assays appeared excellent. Seroreversion was seen in 1 immunocompromised patient. There is little data on antibody kinetics in the immunocompromised with only a handful of published case reports. There were 2 patients in our cohort on rituximab and neither developed detectable antibody. Lack of seroconversion in rituximab and other anti-CD20 agents has been reported elsewhere (Lucchini et al.; Thornton, 2020; Woo et al., 2020b). A study into the immune response in 18 renal transplant patients, which is the largest cohort of immunocompromised patients with confirmed SARS-CoV-2 infection found that 16/18 developed a detectable antibody response (Hartzell et al., 2020). We had 1 renal transplant patient in our cohort who did seroconvert, although with very weak COIs measured and a subsequent reversion

to seronegativity on day 43 postsymptom onset. Overall sensitivity for samples taken from immunocompromised patients using the Roche was low, questioning its utility in this population. It is difficult to draw any definite conclusions about performance in the immunocompromised from these data given the low numbers of patients and samples. In the immunocompromised group, the Abbott assay resulted in numerically higher sensitivities than the Roche, reaching the MHRA sensitivity cut-off for use at  $\geq 21$  days.

Our data set was limited by the samples that were available to use. As we were not collecting samples specifically for this evaluation we were reliant on samples sent to the laboratory for routine clinical reasons. We were further limited by the short period of time serum samples were stored. This may have resulted in some skew to our results of sensitivity using the larger set of samples as a whole as multiple samples were included from each patient with varied and inconsistent time points available for analysis across patients. Drawing definite conclusions about antibody kinetics or the performance of these assays in the immunocompromised is difficult due to low patient numbers, but currently very little data about antibody in the immunocompromised is available and this represents the second largest cohort of immunocompromised patients with antibody data following SARS-CoV-2 infection. Significant further work into the antibody response in the immunocompromised is still needed.

## Authors' contributions

David Harrington performed the primary data analysis and literature search. Tahira Azim performed the testing of all samples. Mark Hopkins contributed some graphical data. All authors were involved in the planning of the project and writing and editing of the manuscript.

## Declaration of competing interests

The authors report no conflicts of interest relevant to this article.

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