



OPEN

The influence of time on the sensitivity of SARS-CoV-2 serological testing

Arturo Torres Ortiz^{1,2}, Fernanda Fenn Torrente^{1,3}, Adam Twigg^{4,5}, James Hatcher⁶, Anja Saso^{4,7,8}, Tanya Lam⁴, Marina Johnson¹, Helen Wagstaffe¹, Rishi Dhillon⁹, Anabelle Lea Mai¹⁰, David Goldblatt¹, Rachel Still¹¹, Matthew Buckland¹⁰, Kimberly Gilmour¹⁰ & Louis Grandjean^{1,4}✉

Sensitive serological testing is essential to estimate the proportion of the population exposed or infected with SARS-CoV-2, to guide booster vaccination and to select patients for treatment with anti-SARS-CoV-2 antibodies. The performance of serological tests is usually evaluated at 14–21 days post infection. This approach fails to take account of the important effect of time on test performance after infection or exposure has occurred. We performed parallel serological testing using 4 widely used assays (a multiplexed SARS-CoV-2 Nucleoprotein (N), Spike (S) and Receptor Binding Domain assay from Meso Scale Discovery (MSD), the Roche Elecsys-Nucleoprotein (Roche-N) and Spike (Roche-S) assays and the Abbott Nucleoprotein assay (Abbott-N) on serial positive monthly samples collected as part of the Co-STARs study (www.clinicaltrials.gov, NCT04380896) up to 200 days following infection. Our findings demonstrate the considerable effect of time since symptom onset on the diagnostic sensitivity of different assays. Using a time-to-event analysis, we demonstrated that 50% of the Abbott nucleoprotein assays will give a negative result after 175 days (median survival time 95% CI 168–185 days), compared to the better performance over time of the Roche Elecsys nucleoprotein assay (93% survival probability at 200 days, 95% CI 88–97%). Assays targeting the spike protein showed a lower decline over the follow-up period, both for the MSD spike assay (97% survival probability at 200 days, 95% CI 95–99%) and the Roche Elecsys spike assay (95% survival probability at 200 days, 95% CI 93–97%). The best performing quantitative Roche Elecsys Spike assay showed no evidence of waning Spike antibody titers over the 200-day time course of the study. We have shown that compared to other assays evaluated, the Abbott-N assay fails to detect SARS-CoV-2 antibodies as time passes since infection. In contrast the Roche Elecsys Spike Assay and the MSD assay maintained a high sensitivity for the 200-day duration of the study. These limitations of the Abbott assay should be considered when quantifying the immune correlates of protection or the need for SARS-CoV-2 antibody therapy. The high levels of maintained detectable neutralizing spike antibody titers identified by the quantitative Roche Elecsys assay is encouraging and provides further evidence in support of long-lasting SARS-CoV-2 protection following natural infection.

Following natural infection or vaccination, sensitive measurement of SARS-CoV-2 serological status is important to identify immune correlates of protection from future waves of the pandemic, evaluate those in need of booster

¹Department of Infection, Inflammation and Immunity, Great Ormond Street Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK. ²Department of Infectious Diseases, Imperial College London, Paddington, London W2 1NY, UK. ³UCL Medical School, University College London, 74 Huntley Street, London WC1E 6DE, UK. ⁴Department of Infectious Diseases, Great Ormond Street Hospital, Great Ormond Street, London WC1N 3JH, UK. ⁵School of Clinical Medicine, University of Cambridge, Cambridge Biomedical Campus, Box 111, Cambridge CB2 0SP, UK. ⁶Department of Microbiology, Great Ormond Street Hospital, Great Ormond Street, London WC1N 3JH, UK. ⁷Department of Tropical and Infectious Diseases, LSHTM, Keppel St, Bloomsbury, London WC1E 7HT, UK. ⁸MRC Gambia at LSHTM, PO Box 273, Fajara, The Gambia. ⁹Public Health Wales Microbiology, University Hospital of Wales, Heath Park Way, Cardiff CF14 4XW, UK. ¹⁰Clinical Immunology, Camelia Botnar Laboratories, Great Ormond Street Hospital, Great Ormond Street, London WC1N 3JH, UK. ¹¹Laboratory Medicine Service Swansea, Bay University Health Board Morriston Hospital, Swansea SA6 6NL, UK. ✉email: l.grandjean@ucl.ac.uk

vaccination and identify candidates for SARS-CoV-2 antibody therapy. The rapid response to the COVID-19 pandemic has led to the development of a wide range of serological tests suitable for evaluating SARS-CoV-2 exposure, infection or vaccination status^{1–3}. Typically, these tests are approved for use by the regulatory authorities based on their performance against a panel of reference sera including positive and negative controls at either 14- or 21-days post infection⁴.

Public Health England reported a 93.9% sensitivity for the Abbott SARS-CoV-2 IgG Nucleoprotein assay⁵ and 100% for the Roche Elecsys Nucleoprotein assay at ≥ 14 days post infection⁶. This led to widespread adoption of these tests across NHS laboratories for testing at population level. Other studies have confirmed this test performance at 14–21 days post infection^{7,8}. Population level serological studies have also based their conclusions—vital to guide national policy—on the basis of these tests⁹ without considering how time since infection influences the performance of the test. The problem with this approach is that it does not take into account SARS-CoV-2 humoral dynamics and changes in avidity over time^{10,11}. Although serological tests with limited diagnostic range may demonstrate excellent sensitivity shortly after infection, it is unclear how they will perform with time following infection or vaccination.

In order to address this question, we applied 4 widely used serological assays in parallel to serial samples from the Co-STARS study¹² in which staff testing seropositive to SARS-CoV-2 were followed for up to 200 days following infection. We compared the proportion of samples that remained seropositive over time using a survival analysis and determined the decay rate of the nucleoprotein (N) antibody and the spike (S) antibody for each test using a previously published mathematical model fitted to the data.

Materials and methods

Study setting and design. Serological testing was performed on stored serum samples collected as part of the Co-STARS study (www.clinicaltrials.gov, NCT04380896), approved by the UK National Health Service Health Research Authority and run at Great Ormond Street Hospital between April 29th and November 2020 in accordance with the relevant guidelines¹⁰. Briefly, Co-STARS was a 1-year single-centre prospective cohort study of antibody responses to COVID-19 infection in healthcare workers. Serum samples were taken from the 3657 participants at baseline and underwent a screening ELISA using the EDI assay. Repeated monthly serum samples were then taken from those with a seropositive baseline screening test for up to 250 days after the date of infection. Written informed consent was obtained from all participants. Those samples identified as seropositive with available symptom start date had further confirmatory testing with the quantitative three antigen MSD assay.

Study participants. The majority of hospital staff were eligible for the Co-STARS study¹². Only those participants with significant immunosuppression, those that had received blood products within 6 months of recruitment and those that had active and ongoing symptoms of SARS-CoV-2 infection (within the last 21 days) were excluded. Only samples from individuals with at least one positive test from any platform were included in the analysis. Moreover, individuals without a known symptom start date were removed.

Data collection. As part of the Co-STARS study all participants undertook a detailed standardised online questionnaire at study entry¹². This included the date of onset of COVID-19 symptoms, and any SARS-CoV-2 diagnostic test results.

Comparison of serological assays. Samples taken as part of the Co-STARS study¹² which had an accompanying symptom start date available for analysis were initially screened for seropositivity by the EDI assay or by any of the three antigens of the Meso Scale Discovery (MSD) assay. The selected samples each underwent testing with 4 serological assays: (1) The Roche Elecsys Anti-SARS-CoV-2 electrochemiluminescence immunoassay (ECLIA) assay detects the nucleocapsid (N) antigen (Roche-N); (2) the Roche Elecsys Anti-SARS-CoV-2 S electrochemiluminescence immunoassay (ECLIA) assay detects the spike (S) antigen (Roche-S); (3) the Abbott Nucleoprotein Chemiluminescent Microparticle Immunoassay (CLMIA) assay detects the nucleocapsid (N) antigen (Abbott-N); (4). All tests were performed as per manufacturer's specifications. The four antigen Meso Scale Discovery (MSD) assay was undertaken at the WHO Pneumococcal Supranational Reference Laboratory at the UCL Institute of Child Health. Only 3 antigens were reported from the MSD assay (the Spike, the Nucleoprotein and the RBD) as the baseline test performance of the N-terminal domain (NTD) antibody response was insufficient for further evaluation as previously reported¹³. The Roche-N and Roche-S assays were undertaken by the Laboratory Medicine Service of Swansea Bay University Health Board, Morriston Hospital, Swansea. The Abbott-N assay was undertaken by Public Health Wales Microbiology at Cardiff and Vale University Hospital. All samples were stored and transported between laboratories at -80°C and only removed for aliquoting prior to testing to avoid unnecessary freeze–thaw cycles.

Statistical analysis and modelling. In order to evaluate the relative proportion of seropositive tests in the parallel serological assays over time, a time-to-event analysis was performed using the time from symptom onset and the first negative test for each assay after a first positive test as the event of interest using the R package *survival*^{14,15}. Only tests taken > 14 days after symptom onset were considered in the analysis. No tests were performed between 14- and 21-days post symptoms, and thus using 14 or 21 days post symptom onset as threshold did not affect our results. A participant was defined as seropositive when at least one of the 4 tests undertaken was seropositive. If the other tests that were run in parallel never became seropositive, the time-to-event was set to the earliest test taken for that individual. If a participant never became seronegative during the follow-up period, a right-censored observation was added at the time of the last serological test.

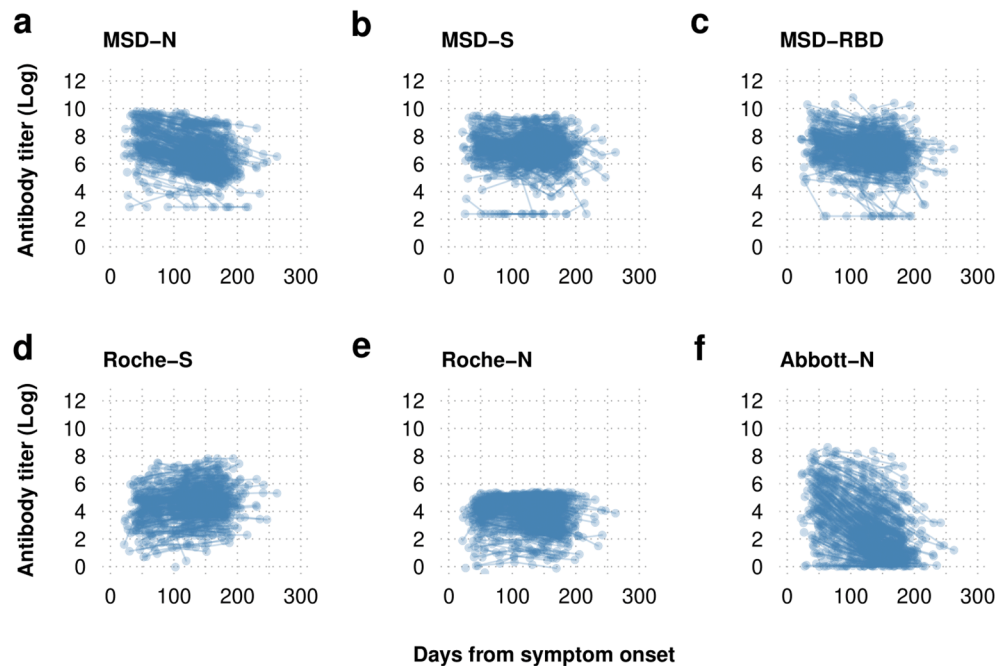


Figure 1. Log transformed serial serological antibody titer data plotted by time from symptom onset. Antibody dynamics are dependent on the assay used with the sensitive Roche-S and MSD-S assay demonstrating maintenance of the spike protein antibody while the nucleoprotein antibody is shown to wane with the MSD and Abbott-N assays but to a lesser extent with the Roche-N assay.

Additionally, the decay rate after 21 days since symptom onset was estimated using a Bayesian generalized linear mixed model as implemented in the R package *MCMCglmm*¹⁶, where time from symptom onset was included as a fixed effect and study participants as a random effect. Therefore, a unique slope for the regression was estimated for the entire population, while the intercept was allowed to vary between the study participants. The decay rate was estimated from the slope of the linear model.

To assess the overall diagnostic capability of the Abbott-N assay, a receiver operating characteristic curve (ROC) analysis was performed using the pROC package within R¹⁷. The MSD-N and Roche-N assays were used as the gold standard for the comparison.

Ethical approval and consent. The study had national Integrated Research Application System (IRAS) approval and all participants in the study provided informed consent.

Results

A total of 950 samples from 329 participants seropositive by any assay after 14 days underwent testing with the Roche-N, Roche-S, the MSD and the Abbott-N assay. The majority of the participants (98%, 321/329) had a positive result by two or more assays.

Antibody decay with time. Plotting the raw log transformed antibody titers over time since symptom onset (Fig. 1) demonstrated that antibody dynamics were dependent on the assay undertaken. The production of spike antibodies was demonstrated to be maintained at high levels up to 200 days when evaluated by the MSD and the quantitative Roche-S assay. All nucleoprotein antibody assays demonstrated decay of the nucleoprotein antibody over time. This was most pronounced in the Abbott-N assay and much less so in the Roche-N assay which demonstrated slow waning of the nucleoprotein antibody.

Assay sensitivity with time post symptom onset. The existing published test performance for all assays undertaken is provided in Table 1. The sensitivity of all assays (at least 14 days from symptom onset) at 50, 100 and 150 days is provided in Table 2. All assays demonstrated a reasonable sensitivity at 50 days following infection (Fig. 2a). As time passed following infection, the Abbott-N assay rapidly became seronegative (Fig. 2a), with a median survival time inferred at 175 days (95% CI 168–185 days), whereas the survival probability at 150 days was inferred to be 95% for the Roche-N (95% CI 0.92–0.97), and 91% for the MSD-N assay (95% CI 0.87–0.94). The Roche-S and MSD-S assays remained seropositive for the duration of the study. The MSD-RBD assay showed some evidence of waning seropositivity over time (90% Survival probability at 150 days, 95% CI 0.88–0.94).

A total of 45% (159/329) of the individuals had a negative result using the Abbott-N assay during the course of the study. For the MSD test, 16% (52/329) of participants had a negative test for the N antigen, 11% (36/329)

Tests	Manufacturer	Target Antigen	Type	Sens [†]	Spec	# Samples/ Patients*	Days post symptom onset
Abbott-N ¹⁸	Abbott-N	Nucleoprotein	CLMIA	100.0% (day 17)	99.9%	689/125	≥ 21
Roche-N ¹⁹	Roche Cobas	Nucleoprotein	ECLIA	99.5%	99.8%	496/102	≥ 14
Roche-S ²⁰	Roche Cobas	Spike protein	ECLIA	96.6%	100%	1485 [‡] /331	≥ 15
MSD ¹³	Meso Scale Discovery	Nucleoprotein	ECLIA	87.2%	92.8%	196/196	≥ 21
		Spike protein		97.9%	97.4%	47/47	≥ 21
		RBD (receptor binding domain)		93.6%	92.3%	47/47	≥ 21

Table 1. A summary of the existing published data for the commercially available tests in this comparison. The corresponding antigen target and published sensitivity, specificity, positive predictive value and negative predictive value (if available). *ECLIA* Electro Chemiluminescent Immunoassay, *CLMIA* chemiluminescent microparticle immuno assay, *PPV* positive predictive value, *NPV* negative predictive value, *Sens* sensitivity, *Spec* specificity, *ELISA* enzyme linked immunosorbent assay. [†]The highest reported sensitivity. [‡]233 of these samples were tested at ≥ 15 days post PCR diagnosis. *For sensitivity testing.

	Survival probability (95% CI)		
	50-day	100-day	150-day
Abbott-N	0.985 (0.97;0.99)	0.919 (0.89;0.95)	0.655 (0.6;0.71)
Roche-N	0.988 (0.98;1.0)	0.963 (0.94;0.98)	0.949 (0.92;0.97)
Roche-S	0.991 (0.98;1.0)	0.966 (0.95;0.99)	0.952 (0.93;0.98)
MSD			
N	0.988 (0.98;1.0)	0.972 (0.95;0.99)	0.907 (0.87;0.94)
S	0.997 (0.99;1.0)	0.978 (0.96;0.99)	0.968 (0.95;0.99)
RBD	0.994 (0.99;1.0)	0.969 (0.95;0.99)	0.909 (0.88;0.94)

Table 2. Sensitivity of compared assays at 50, 100 and 150 days from symptom onset.

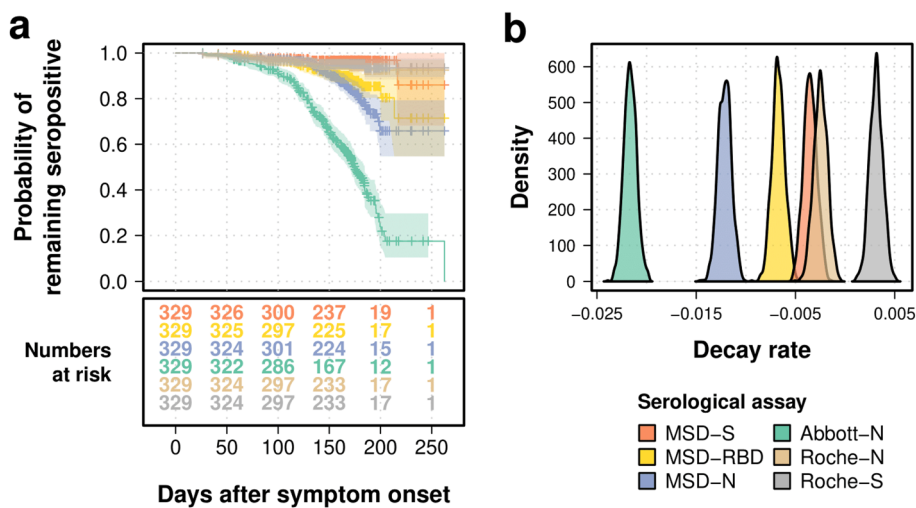


Figure 2. Comparison of seropositivity and antibody dynamics between serological tests. The Roche-S assay target the spike antibody, the Abbott-N and the Roche-N assays target the N-antibody while the MSD assay targets the N-, the S- and the antibody to the Receptor Binding Domain (RBD) of the spike protein in parallel. (a) Kaplan–Meier curve and numbers at risk (the number of participants under follow up with serological tests available for analysis at that time point) for different serological tests. Y-axis represents the probability of remaining seropositive, while the X-axis shows days after symptom onset with numbers of participants under follow up shown in the table below. (b) Inferred posterior density distributions of the decay rate in a generalized linear mixed model.

	Mean	95% CI
MSD-N	-0.0121	-0.0134; -0.0107
MSD-RBD	-0.0068	-0.008; -0.0055
MSD-S	-0.0035	-0.0048; -0.0023
Abbott-N	-0.0216	-0.0229; -0.0204
Roche-S	0.0031	0.0018; 0.0044
Roche-N	-0.0025	-0.0039; -0.0012

Table 3. Decay rate for each serological assay (log arbitrary units per day) estimated in a generalized linear mixed model.

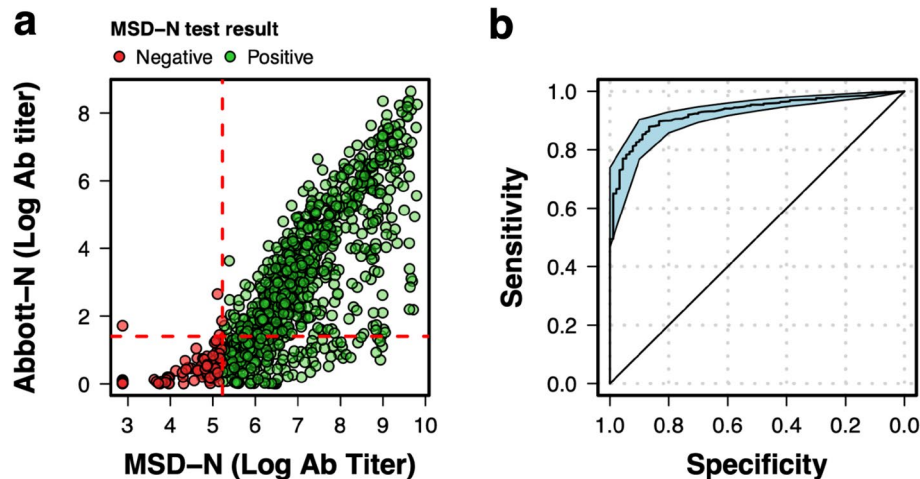


Figure 3. Comparison of antibody titers between the Abbott-N assay and the MSD-N assay. (a) The quantitative results for the MSD-N assay were compared to those of the Abbott-N test for each sample taken. Colours divide the samples depending on whether it was positive (green) or negative (red) for the MSD-N assay. Dotted red lines represent the seropositivity threshold for the Abbott-N assay (horizontal) and the MSD-N test (vertical). (b) ROC curve for the Abbott-N assay using the MSD-N test as gold standard. The x~y line represents the profile of a random classifier. Blue shaded area shows the 95% CI.

for RBD, and 3% (11/329) for the S antigen. For the Roche platform, 5.5% (18/329) of the individuals had a negative result with the Roche-N assay, while only 4.8% (16/329) of them had a negative result for the S antigen over the course of the study.

Mathematical model fits to estimate antibody decay. To estimate the decay rate for each antibody and assay studied, a generalized linear mixed model was fitted to the trajectory of antibody decay after 21 days from symptom onset, where the decay rate was estimated as the slope of the antibody titer through time. Under the most sensitive and quantitative Roche -S assay the spike antibody demonstrated no decay at all and rather a slow rate of increased titers over time from symptom onset (0.0031, 95% CI 0.0018–0.0044, Fig. 2b). In accordance with the raw observed data, all nucleoprotein antibodies under the mathematical model decayed. This was most pronounced in the Abbott-N assay (-0.022, 95% CI -0.023 to -0.02) and least pronounced in the Roche -N assay (-0.0025, 95% CI -0.0039 to -0.0012, Fig. 2b, Table 3).

The lower performance of the Abbott-N assay can be explained by a lower detection of titer values as their concentration wanes over time. When compared to the quantitative MSD-N, 26% (222/860) of all positive samples by the MSD-N were negative for the Abbott-N test (Fig. 3a). A total of 75% of samples (137/183) positive by the MSD-N with an MSD arbitrary titer value lower than 403 were negative for the Abbott-N assay. Using the currently manufacturer recommended threshold of 1.4 arbitrary units, the Abbott-N test was characterized by a high specificity of 0.96 and a sensitivity of 0.74 using all our test results after 14 days. Using a ROC curve (Fig. 3b), the optimal cut-off that maximises both specificity and sensitivity was estimated to be 0.845 arbitrary units.

Discussion

Sensitive measurement of SARS-CoV-2 seropositivity is key to evaluate who has been infected or exposed to SARS-CoV-2, to determine the correlates of protection from future disease, stratify those that need booster vaccination and target the use of anti-SARS-CoV-2 antibodies to those that are seronegative. To our knowledge no other study has evaluated the sensitivity of multiple diagnostic tests in parallel on longitudinally collected serological samples. This study demonstrates that as time elapses after infection, the sensitivity of serological

testing varies widely depending on the test used. Although serological tests may be demonstrated to perform well 14–21 days after infection, this initial test performance often diminishes as time passes. In order to evaluate whether or not the population maintains SARS-CoV-2 antibodies it is vital that we utilize serological tests that remain sensitive over time.

Initial published baseline test performance reports concluded that the Abbott-N assay was a high-performance test and a key tool in SARS-CoV-2 surveillance¹⁸. Our data demonstrate that as time passes following infection the sensitivity of this assay declines rapidly until at < 6 months following infection it is no more than 50% sensitive. Our findings support the concerns raised by others regarding the poor performance of some nucleoprotein based assays^{21,22}.

In contrast, the Roche assays, particularly the Roche Elecsys Anti-SARS-CoV-2 Spike assay maintained high sensitivity for the 200-day duration of the study. Although there remains no single correlate of sterilizing or protective immunity following SARS-CoV-2 infection or vaccination, it is clear that natural infection and the presence of neutralizing spike antibodies decreases the possibility of re-infection and the severity of disease upon re-exposure to currently circulating strains²³. Our finding that spike antibodies remained at high titers 200 days after infection adds to our previous study on this topic¹⁰ and provides further evidence in support of long-lasting protection against severe disease from currently circulating strains. Fitting mathematical models to the raw data of the Roche spike assay demonstrated that spike antibody titers did not decay but rather increased slightly over the duration of the study. The Roche nucleoprotein assay also maintained sensitivity for the duration of the study with a low rate of decay. Although this assay is semi-quantitative, our findings suggest that this could be used to sensitively identify those that have been vaccinated from those that have been both vaccinated and infected.

Many studies have evaluated the impact of time on test sensitivity over the first 3 weeks following symptom onset^{24–26}. However, we found no other study that had examined the sensitivity of antibody testing on parallel longitudinal samples collected between 1 and 6 months after infection or exposure. Assays with a higher titer cut-off for detection may perform well in the initial period after infection, but fail to detect seropositivity as antibody levels wane over time. We show that the Abbott-N test failed to detect 75% of samples positive for the MSD-N with a titer value lower than 403, which makes the Abbott-N assay less suitable for seroprevalence studies. Using a ROC curve and the MSD-N and Roche-N assays as the gold standard, we showed that a lower threshold of 0.845 instead of 1.4 arbitrary units may be more suitable to optimize the sensitivity and specificity. Even though different thresholds may be relevant depending on whether sensitivity or specificity needs to be prioritized, our findings suggest that the high Abbott-N test threshold results in a high number of false negatives. These findings are concordant with previous reports showing a range of high uncertainty between 0.49 and 1.4²⁷. Barzin et al.²⁸ used Abbott-N testing alone to determine SARS-CoV-2 seroprevalence in 2,973 asymptomatic out-patients in North Carolina estimating a seroprevalence of 0.8%. Similarly, Wilkins et al.²⁹ used Abbott-N on 6510 healthcare workers up to 150 days after symptom onset and estimated a seroprevalence of 4.8%. Our findings suggest that previously published surveys of SARS-CoV-2 seroprevalence such as these could have significantly underestimated the true prevalence of SARS-CoV-2 humoral immunity.

Memory T-cell interferon gamma release or proliferation assays in response to SARS-CoV-2 antigens provide an alternative means of assessing prior exposure to infection. However, these assays are limited by cross reactive immunity to the seasonal coronaviruses decreasing specificity^{30,31}.

Although all serological tests used in the study demonstrated a high initial specificity, one limitation is that only 38% of participants had a confirmatory SARS-CoV-2 PCR result. Our data may therefore be influenced by an unknown proportion of falsely positive serological tests. However, at entry to the study, all seropositive participants had both a screening EDI nucleoprotein assay and an MSD assay performed which limited the chances of a falsely positive result due to a single erroneous test. Not all samples were processed at the same time; the Roche and Abbott-N assays were processed 3 months after the MSD assays. Despite this, we believe that sample storage and freeze-thawing cycles are unlikely to have influenced our findings as the Roche quantitative spike assay was performed last and demonstrated the highest prolonged levels of spike antibody of all tests used.

In summary, although serological tests may demonstrate high sensitivity 3-weeks after SARS-CoV-2 infection, this is far from the case with some tests 6-months after infection. The Abbott-N assay performed poorly at this time, whereas the Roche and MSD tests maintained a high sensitivity for the 200 days of the study. Tests that perform poorly over time will lead to spurious estimates in population level seroprevalence studies and findings from these studies should be adjusted to account for sensitivity of the test used and the time since infection. Test performance as time passes post infection should be considered before evaluating who is a candidate for booster vaccination or anti-SARS-CoV-2 antibody therapy.

Data availability

The dataset used during the current study is available as Supplementary Data File 1.

Received: 22 January 2022; Accepted: 6 June 2022

Published online: 22 June 2022

References

1. Lisboa Bastos, M. *et al.* Diagnostic accuracy of serological tests for covid-19: Systematic review and meta-analysis. *BMJ* <https://doi.org/10.1136/bmj.m2516> (2020).
2. Kubina, R. & Dziedzic, A. Molecular and serological tests for COVID-19. A comparative review of SARS-CoV-2 coronavirus laboratory and point-of-care diagnostics. *Diagnostics* **10**, 434. <https://doi.org/10.3390/diagnostics10060434> (2020).
3. la Marca, A. *et al.* Testing for SARS-CoV-2 (COVID-19): A systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. *Reprod. Biomed. Online* **41**, 483–499. <https://doi.org/10.1016/j.rbmo.2020.06.001> (2020).

4. Cheng, M. P. *et al.* Serodiagnostics for severe acute respiratory syndrome-related coronavirus 2: A narrative review. *Ann. Intern. Med.* **173**, 450–460. <https://doi.org/10.7326/M20-2854> (2020).
5. Public Health England. *Evaluation of the Abbott SARS-CoV-2 IgG for the Detection of Anti-SARSCoV-2 Antibodies*. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/890566/Evaluation_of_Abbott_SARS-CoV-2_IgG_PHE.pdf (2020).
6. Public Health England. *Evaluation of Roche Elecsys AntiSARS-CoV-2 Serology Assay for the Detection of Anti-SARS-CoV-2 Antibodies*. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/891598/Evaluation_of_Roche_Elecsys_anti_SARS-CoV-2_PHE_200610_v8.1_FINAL.pdf (2020).
7. Coste, A. T., Jaton, K., Papadimitriou-Olivgeris, M., Greub, G. & Croxatto, A. Comparison of SARS-CoV-2 serological tests with different antigen targets. *J. Clin. Virol.* **134**, 104690. <https://doi.org/10.1016/j.jcv.2020.104690> (2021).
8. Ainsworth, M. *et al.* Performance characteristics of five immunoassays for SARS-CoV-2: A head-to-head benchmark comparison. *Lancet Infect. Dis* [https://doi.org/10.1016/S1473-3099\(20\)30634-4](https://doi.org/10.1016/S1473-3099(20)30634-4) (2020).
9. Pollán, M. *et al.* Prevalence of SARS-CoV-2 in Spain (ENE-COVID): A nationwide, population-based seroepidemiological study. *The Lancet* **396**, 535–544. [https://doi.org/10.1016/S0140-6736\(20\)31483-5](https://doi.org/10.1016/S0140-6736(20)31483-5) (2020).
10. Grandjean, L. *et al.* Long-term persistence of spike protein antibody and predictive modeling of antibody dynamics after infection with severe acute respiratory syndrome coronavirus 2. *Clin. Infect. Dis.* <https://doi.org/10.1093/cid/ciab607> (2021).
11. Bauer, G. *et al.* The challenge of avidity determination in SARS-CoV-2 serology. *J. Med. Virol.* **93**, 3092–3104. <https://doi.org/10.1002/jmv.26863> (2021).
12. Great Ormond Street Hospital for Children NHS Foundation Trust. *COVID-19 Staff Testing of Antibody Responses Study (CO-STARS)*. <https://clinicaltrials.gov/ct2/show/NCT04380896> (2021).
13. Johnson, M. *et al.* Evaluation of a novel multiplexed assay for determining IgG levels and functional activity to SARS-CoV-2. *J. Clin. Virol.* **130**, 104572. <https://doi.org/10.1016/j.jcv.2020.104572> (2020).
14. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2018).
15. Therneau, T. M. & Grambsch, P. M. *Modeling Survival Data: Extending the Cox Model* (Springer, 2000).
16. Hadfield, J. D. MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *J. Stat. Softw.* **33**, 1–22. <https://doi.org/10.18637/jss.v033.i02> (2010).
17. Robin, X. *et al.* pROC: An open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinform.* **12**, 1–8. <https://doi.org/10.1186/1471-2105-12-77> (2011).
18. Bryan, A. *et al.* Performance characteristics of the abbot architect sars-cov-2 igg assay and seroprevalence in Boise, Idaho. *J. Clin. Microbiol.* <https://doi.org/10.1128/JCM.00941-20> (2020).
19. Muench, P. *et al.* Development and validation of the Elecsys anti-SARS-CoV-2 immunoassay as a highly specific tool for determining past exposure to SARS-CoV-2. *J. Clin. Microbiol.* <https://doi.org/10.1128/JCM.01694-20> (2020).
20. FDA. Elecsys Anti-SARS-CoV-2 S - Instructions for Use 2021. <https://www.fda.gov/media/144037>.
21. Rosadas, C., Randell, P., Khan, M., McClure, M. O. & Tedder, R. S. Testing for responses to the wrong SARS-CoV-2 antigen?. *The Lancet* **396**, e23. [https://doi.org/10.1016/S0140-6736\(20\)31830-4](https://doi.org/10.1016/S0140-6736(20)31830-4) (2020).
22. Bolotin, S. *et al.* SARS-CoV-2 seroprevalence survey estimates are affected by anti-nucleocapsid antibody decline. *J. Infect. Dis.* <https://doi.org/10.1093/infdis/jiaa796> (2021).
23. Hall, V. J. *et al.* SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: A large, multicentre, prospective cohort study (SIREN). *The Lancet* **397**, 1459–1469. [https://doi.org/10.1016/S0140-6736\(21\)00675-9](https://doi.org/10.1016/S0140-6736(21)00675-9) (2021).
24. Whitman, J. D. *et al.* Evaluation of SARS-CoV-2 serology assays reveals a range of test performance. *Nat. Biotechnol.* <https://doi.org/10.1038/s41587-020-0659-0> (2020).
25. Piec, I. *et al.* Performance of SARS-CoV-2 serology tests: Are they good enough?. *PLoS ONE* **16**, e0245914. <https://doi.org/10.1371/journal.pone.0245914> (2021).
26. Wang, H., Ai, J., Loeffelholz, M. J., Tang, Y. W. & Zhang, W. Meta-analysis of diagnostic performance of serology tests for COVID-19: Impact of assay design and post-symptom-onset intervals. *Emerg. Microbes Infect.* <https://doi.org/10.1080/22221751.2020.1826362> (2020).
27. Castro, M. D. M. *et al.* Performance verification of the Abbott SARS-CoV-2 test for qualitative detection of IgG in Cali, Colombia. *PLoS ONE* **16**, e0256566. <https://doi.org/10.1371/journal.pone.0256566> (2021).
28. Barzin, A. *et al.* SARS-CoV-2 seroprevalence among a Southern U.S. population indicates limited asymptomatic spread under physical distancing measures. *MBio* <https://doi.org/10.1128/mBio.02426-20> (2020).
29. Wilkins, J. T. *et al.* Seroprevalence and correlates of SARS-CoV-2 antibodies in health care workers in Chicago. *Open Forum Infect. Dis.* <https://doi.org/10.1093/ofid/ofaa582> (2021).
30. le Bert, N. *et al.* SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* <https://doi.org/10.1038/s41586-020-2550-z> (2020).
31. Lipsitch, M., Grad, Y. H., Sette, A. & Crotty, S. Cross-reactive memory T cells and herd immunity to SARS-CoV-2. *Nat. Rev. Immunol.* <https://doi.org/10.1038/s41577-020-00460-4> (2020).

Acknowledgements

We would like to dedicate this article to the staff members who died of COVID-19 at Great Ormond Street Hospital during the first wave of the pandemic. We would also like to thank all the staff at Great Ormond Street Hospital who have taken part in the study. In addition, we are very grateful for all the hard work undertaken by the Great Ormond Street rapid response and microbiology laboratory staff and the staff in the immunology laboratories both in the Camelia Botnar Laboratory and the Great Ormond Street Institute of Child Health who ensured that all the PCR tests and serological assays were completed in a timely manner. The authors would also like to thank the biomedical scientists and colleagues in the affiliated laboratories for their hard work in running the samples and collating the results. RD would like to thank Linda Tyson and Jon Evans of the Wales National Virology Centre, PHW Microbiology Cardiff. RS would like to thank Non Gwynn, Jenna Walters, James Murphy and colleagues in Laboratory Medicine, Morrision Hospital, Swansea.

Author contributions

The study was designed and implemented by: L.G., A.T.O., J.H., R.D., R.S., D.G. and K.G. Testing for SARS-CoV-2 RNA and antibody was conducted by: L.G., F.T., A.T., A.L.M., K.G., J.H., A.S., T.L., M.J., H.W., M.B., R.D., R.S. and D.G. Data analysis was carried out by A.T.O. and L.G. The first draft of the manuscript was prepared by: A.T.O., F.T. and L.G., with input from all co-authors.

Funding

LG was supported by the Wellcome Trust (201470/Z/16/Z), the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number 1R01AI146338, the GOSH Charity (VC0921) and the GOSH/ICH Biomedical Research Centre (www.nihr.ac.uk). AS was supported by the Wellcome Trust (220565/Z/20/Z). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-022-14351-2>.

Correspondence and requests for materials should be addressed to L.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2022