



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Original article

Clinical evaluation of serological IgG antibody response on the Abbott Architect for established SARS-CoV-2 infection

K.L. Chew^{1,*}, S.S. Tan¹, S. Saw¹, A. Pajarillaga¹, S. Zaine¹, C. Khoo¹, W. Wang¹, P. Tambyah², R. Jureen¹, S.K. Sethi¹¹ Department of Laboratory Medicine, National University Hospital, Singapore² Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

ARTICLE INFO

Article history:

Received 20 May 2020

Received in revised form

30 May 2020

Accepted 31 May 2020

Available online 9 June 2020

Editor: F. Allerberger

Keywords:

Abbott Architect

COVID-19

Immunoglobulin G

SARS-CoV-2

Serology

ABSTRACT

Objective: This study aimed to evaluate the diagnostic performance of the Abbott Architect SARS-CoV-2 IgG assay in COVID-19 patients.**Methods:** Residual sera from 177 symptomatic SARS-CoV-2-positive patients and 163 non-COVID-19 patients were tested for antibody with the Abbott SARS-CoV-2 IgG assay (Abbott Diagnostics, Chicago, USA). Clinical records for COVID-19 patients were reviewed to determine the time from onset of clinical illness to testing.**Results:** Specificity of the assay was 100.0% (95%CI: 97.1–100.0%). The clinical sensitivity of the assay varied depending on time from onset of symptoms, increasing with longer periods from the onset of clinical illness. The clinical sensitivity at ≤6 days was 8.6% (7/81; 95%CI: 3.8–17.5%), at 7–13 days 43.6% (17/39; 95%CI: 28.2–60.2%), at 14–20 days 84.0% (21/25; 95%CI: 63.1–94.7%), and at ≥21 days 84.4% (27/32; 95%CI: 66.5–94.1%). Clinical sensitivity was higher in the ≥14-day group compared to <14 days. There were no differences between the 14–20-day and ≥21-days groups; the combined clinical sensitivity for these groups (≥14 days) was 84.2% (49/57; 71.6–92.1%).**Conclusion:** The Abbott SARS-CoV-2 IgG test has high specificity. Clinical sensitivity was limited in the early stages of disease but improved from 14 days after the onset of clinical symptoms. **K.L. Chew, Clin Microbiol Infect 2020;26:1256.e9–1256.e11**

© 2020 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Introduction

Coronavirus disease 2019 (COVID-19) continues to spread globally, and laboratory confirmation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is of paramount importance in the efforts to combat this pandemic. Testing and turnaround time may vary from a few hours to days, depending on the workload and throughput of analysers in operation.

There is interest in developing other testing modalities for the confirmation of diagnosis, such as antigen testing and serological assays [1]. The SARS-CoV-2 antibody response may involve immunoglobulin A (IgA), immunoglobulin M (IgM), and/or immunoglobulin G (IgG). Long and colleagues have reported that, in a

cohort of 70 patients, the proportion of patients with a positive IgG reached 100% 17–19 days after the onset of symptoms, as tested with a magnetic chemiluminescence enzyme immunoassay [2]. Nevertheless, questions remain regarding the clinical utility of serological testing as a diagnostic tool. In this study we aimed to test the Abbott Architect SARS-CoV-2 IgG on our Abbott Architect i4000SR analyser, which has a throughput of 400 tests/hour. Residual sera from a cohort of SARS-CoV-2-positive COVID-19 patients were utilized for testing, and we established the clinical sensitivity and specificity of the assay in determining the utility of IgG detection for SARS-CoV-2.

Methods

We prospectively identified confirmed COVID-19 patients presenting at and admitted to our institution from 30th March 2020 to 15th May 2020. Patients were selected on the basis of a positive

* Corresponding author. K.L. Chew, National University Hospital, 5 Lower Kent Ridge Road, 11907, Singapore.

E-mail address: ka_lip_chew@nuhs.edu.sg (K.L. Chew).

SARS-CoV-2 rRT-PCR from a respiratory sample. Residual sera were collected from this cohort and stored for serological testing. Samples were handled in strict accordance with the WHO biosafety precautions as previously described [3]. Two PCR assays were used during this time period (Fortitude, MirXES, Singapore, and cobas® SARS-COV-2, Roche Diagnostics, USA). Only unique or non-duplicate patients were included and were stratified into four groups based on day of illness (≤ 6 days, 7–13 days, 14–20 days, and ≥ 21 days). Clinical records for COVID-19 patients were reviewed to determine the time from onset of clinical illness to testing. All patients had respiratory symptoms and/or fever. Patients who were asymptomatic at the time of PCR testing for contact screening purposes could not be stratified according to time from onset of illness and were excluded. Negative controls were samples taken from patients prior to December 2019. These included patients with and without other positive serological tests: anti-extractable nuclear antigen antibodies (nine), anti-glomerular basement membrane antibodies (four), anti-smooth muscle antibody (three), hepatitis A IgM (three), Epstein–Barr virus IgM (three), anti-intrinsic factor (five), cytomegalovirus IgM (four), cytomegalovirus IgG (three), syphilis *Treponema pallidum* antibody (five), hepatitis B E antigen (two), Epstein–Barr virus IgA (seven), *Lep-tospira* IgM (three), hepatitis C (nine), hepatitis B surface antigen (seven), anti-double-stranded DNA (three), rubella IgM (four), antinuclear antibodies (ANA) (three), hepatitis A IgG (three), dengue IgG (one), varicella zoster IgM (one), human immunodeficiency virus (eight), and varicella zoster virus IgG (six). All samples were collected in Serum Separator Tubes (Beckton Dickinson, New Jersey, USA) and Abbott SARS-CoV-2 IgG assay was performed on the Abbott Architect i4000SR (Abbott Diagnostics, Chicago, USA) as per the manufacturer's instructions. The assay is a chemiluminescent immunoassay which detects IgG raised against the nucleocapsid protein of SARS-CoV-2. A signal/cut-off (S/CO) ratio of ≥ 1.4 was interpreted as reactive and an S/CO ratio of < 1.4 was interpreted as non-reactive. Calibration was performed and positive quality control (QC) S/CO 1.65–8.40 and negative quality control S/CO ≤ 0.78 were fulfilled prior to analyses of patient samples. Within-day imprecision assessment was performed using QC material. Clinical sensitivity was determined using the SARS-CoV-2 PCR as the reference standard. For determining clinical specificity and cross-reactivity, negative samples collected prior to December 2019 were assumed to be negative as SARS-CoV-2 was first identified late in 2019, and the first patients in Singapore were identified in January 2020. Further analyses of sensitivity and specificity were performed by varying the S/CO ratio cut-offs. Results were analysed using Microsoft Excel 2018 and the Statistical Package for Social Sciences (SPSS). Our study was reviewed and approved by the National Healthcare Group Domain Specific Review Board which provides ethical review for our institution (NHG ROAM Reference Number: 2020/00337).

Results

Within-day imprecision using negative QC material ($n = 20$) on the Architect i4000SR had a mean S/CO of 0.059–0.060, with a coefficient of variation (CV) of 0.0–5.4%. Positive QC material ($n = 20$) had a mean S/CO of 3.332–3.432, with a CV of 0.9–2.9%. This was within the manufacturer's CV claims of 1.1–5.9%.

A total of 177 samples from COVID-19 patients and 163 samples from non-COVID-19 patients were included. All 163 samples from non-COVID-19 patients were negative; the clinical specificity of the assay was 100.0% (95%CI: 97.1–100.0%). The clinical sensitivity of the assay varied depending on the time from onset of symptoms, increasing with longer periods from the onset of clinical illness. The clinical sensitivity at ≤ 6 days was 8.6% (7/81; 95%CI: 3.8–17.5%),

7–13 days 43.6% (17/39; 95%CI: 28.2–60.2%), 14–20 days 84.0% (21/25; 95%CI: 63.1–94.7%), and ≥ 21 days 84.4% (27/32; 95%CI: 66.5–94.1%). Clinical sensitivity was higher in the ≥ 14 -day group compared to < 14 days. There were no differences between the 14–20-days and ≥ 21 -days group; the combined clinical sensitivity for these groups (≥ 14 days) was 84.2% (49/57; 71.6–92.1%).

The S/CO values are summarized in Table 1. The maximum S/CO detected for negative cases was 0.70. The clinical sensitivity was calculated again using S/COs of 1.0 and 0.8 for classifying reactive versus non-reactive results (summarized in Table 2). At a cut-off of 1.0, additional cases would be interpreted as reactive in the ≥ 21 -day group (two samples), 7–13-days group (two samples), and ≤ 6 -days group (one sample). At a cut-off of 0.8, additional cases would be interpreted as reactive in the ≥ 21 -day group (two samples), 7–13-days group (three samples), and ≤ 6 -days group (one sample). A one-way analysis of variance (ANOVA) was conducted to compare the mean levels of SARS-CoV-2 IgG S/CO between groups, and this showed a significant difference amongst the different days of symptoms for the positive COVID-19 patients ($F(3,173) = 41.19$, $p < 0.001$). Post-hoc comparisons using the Tukey honest significant test (HST) indicated that there was no significant difference ($p = 0.976$) in mean S/CO IgG levels in patients at ≥ 21 days (mean = 5.02, SD = 2.90) as compared to patients at 14–20 days of symptoms (mean = 5.29, SD = 2.71). Nevertheless, the mean S/CO values were significantly lower in the 7–13-days group ($p < 0.001$) and ≤ 6 -days group ($p < 0.001$) as compared to the 14–20-days group. This implies that 14 days of symptoms is a suitable time-point to consider testing for SARS-CoV-2 IgG antibodies, and that any fewer days of symptoms would result in significantly lower S/COs, and false-negative results are more likely.

Discussion

Our results demonstrated limited clinical sensitivity of the IgG assay, particularly in the earlier stages of COVID-19 illness. IgG testing is not suitable for laboratory diagnosis in acute disease, but is considered for retrospective testing for epidemiological purposes, if there is suspicion of false-positive or false-negative PCR results, or for contact-tracing purposes. Although high technical specificity was demonstrated, clinicians should be aware of the limitations of IgG testing in interpreting the results. A positive test is an indication of a previous infection, but the timing of the infection—whether it had occurred recently or in the distant past—cannot be determined. Avidity testing is used in other contexts, but currently there are no data in the context of SARS-CoV-2.

Table 1
Summary signal/cut-off (S/CO)

Patient group	Average S/CO (95%CI)	Minimum	Maximum
≥ 21 days	5.02 (4.01–6.02)	0.02	9.64
14–20 days	5.29 (4.22–6.35)	0.03	8.88
7–13 days	2.74 (1.70–3.79)	0.02	9.31
≤ 6 days	0.49 (0.18–0.80)	0.01	6.80
Negative cases	0.07 (0.05–0.08)	0.01	0.70

Table 2
Clinical sensitivity with alternate cut-off for determining reactivity

Sample group	Sensitivity (95%CI)	
	Cut-off of 1.0	Cut-off of 0.8
≥ 21 days	90.6% (73.8–97.5%)	90.6% (73.8–97.5%)
14–20 days	84.0% (63.1–94.8%)	84.0% (63.1–94.8%)
7–13 days	48.7% (32.7–65.0%)	51.3% (35.0–67.3%)
≤ 6 days	9.9% (4.7–19.0%)	9.9% (4.7–19.0%)

As time goes by, the clinical specificity may continue to decline as more patients are infected and develop baseline antibodies against the virus. The utility of IgG testing may thus be further limited. In acute settings, adjudication of potential false results with an alternative molecular test may be a better option than serological testing.

In our study, our sample size encompassed a diverse multi-ethnic population from South-East Asia, thus reducing bias. We also included samples reactive to multiple viruses and autoimmune disorders as negative controls; these were collected prior to the SARS-CoV-2 pandemic, serving to validate potential cross-reactivity. We confirmed that the Abbott SARS-CoV-2 exhibited an excellent specificity, corroborating earlier reports [4]. A limitation in our sample set is that sera from patients with known infection with the other commonly circulating human coronaviruses were unavailable. Future evaluations of serological assays should include such samples where available.

We have shown that the S/CO of SARS-CoV-2 IgG increased over time, with better clinical sensitivity demonstrated between samples that were taken ≥ 14 days compared to < 14 days after the onset of clinical illness. This was statistically significant, but no appreciable differences in S/CO values were otherwise detected between 14–20 days and ≥ 21 days. This finding underscores the important point that, for SARS-CoV-2 IgG, samples taken 14 days after illness may thus serve as an adequate threshold for testing to optimize retrospective identification of COVID-19 patients. Further evaluations should be performed to confirm these findings and to develop a diagnostic algorithm integrating serological testing with other testing modalities.

A number of cases remained negative even after ≥ 14 days after the onset of illness. In the light of progressively increasing S/CO and high specificity of the assay, we propose that modifications to the cut-off for determining reactivity may be considered. In Table 2, lowering the cut-off to 1.0 or 0.8 resulted in improved clinical sensitivity of the assay without compromising specificity in our dataset. However, lowering cut-offs for specific assays should be considered carefully as they may also lower specificity, as has been demonstrated by the analysis of borderline results by Tang et al. [4]. A grey zone or indeterminate range for the assay may be useful in indicating that previous SARS-CoV-2 infections cannot be excluded. So far, cross-reactivity to commonly circulating human coronaviruses has not been demonstrated [5]. Cross-reactivity may occur in SARS and MERS coronavirus patients, but widespread community spread of these strains is uncommon [6].

A correlation between antibody production and outcome or long-term immunity has not been established. There have been media reports of possible reinfection with SARS-CoV-2 after recovery, although there are still questions about the testing methods used, and to date there are no published data documenting reinfections. Animal studies suggest that reinfection is unlikely [7]. Further work is required to determine whether the presence of neutralizing antibodies confers long-term immunity in humans. This is vital for several reasons, including identifying potential donors of convalescent plasma, measuring immunogenicity in vaccine development, and determining the degree of herd immunity in the community [8]. Additional studies are also required to correlate serological studies with clinical outcomes and long-term immunity.

One advantage of the Abbott SARS-CoV-2 IgG assay is that it is performed on the Abbott Architect i4000SR analyser, which has a high throughput and allows for population-based screening of large numbers of patients. The fact is that many patients may be

asymptomatic throughout the infection period or have such mild symptoms that they do not come forward for medical review and subsequent testing. Epidemiological and seroprevalence studies may be too difficult and impracticable to conduct otherwise with testing platforms that have limited throughput.

In conclusion, the Abbott SARS-CoV-2 IgG test has high technical specificity. There is potential room for improving clinical sensitivity of the assay to detect previous infection. Testing of IgG after 14 days from the onset of illness may be considered for retrospective identification of patients. It should be noted, however, that the interpretation of IgG testing may change as more patients develop baseline antibodies against the virus. SARS-CoV-2 IgG testing instead could have multiple other potential applications, particularly if it can be correlated with immunity to subsequent infections. This would be instrumental in vaccine development, clinical trials, and epidemiological studies.

Author contributions

KLC and SST contributed equally in the capacity of first authors. KLC and ST designed the study, performed data curation and analysis, and drafted the manuscript. ST, SSaw, AP, SZ, CK, and WW performed the administration and experiments in the project. SSaw, PT, RJ, and SSethi provided supervision and revision of final manuscript.

Transparency declaration

PT has received grants paid to the National University Hospital from Roche (CP40617, MV40618), Johnson & Johnson (63623872FLZ3001, 63623872FLZ2002), Sanofi Pasteur (H-030-014), GlaxoSmithKline, and Shionogi (1601T0831). All other authors have no conflicts of interest to declare. No external funding was received for this study.

Acknowledgements

We would like to thank Temasek Holdings Pte Ltd for sponsoring the laboratory testing kits used in this study.

References

- [1] Zhai P, Ding Y, Wu X, Long J, Zhong Y, Li Y. The epidemiology, diagnosis and treatment of COVID-19. *Int J Antimicrob Agents* 2020;55:105955.
- [2] Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020. <https://doi.org/10.1038/s41591-020-0897-1> [Online Ahead of Print].
- [3] Tan SS, Yan B, Saw S, Lee CK, Chong AT, Jureen R, et al. Practical laboratory considerations amidst the COVID-19 outbreak: early experience from Singapore. *J Clin Pathol* 2020. <https://doi.org/10.1136/jclinpath-2020-206563> [Online Ahead of Print].
- [4] Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical performance of two SARS-CoV-2 serologic assays. *Clin Chem* 2020. <https://doi.org/10.1093/clinchem/hvaa120> [Online Ahead of Print].
- [5] Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med* 2020. <https://doi.org/10.1038/s41591-020-0913-5> [Online Ahead of Print].
- [6] Okba NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. *Emerg Infect Dis* 2020;26. <https://doi.org/10.3201/eid2607.200841>.
- [7] Bao L, Deng W, Gao H, Xiao C, Liu J, Xue J, et al. Lack of reinfection in rhesus macaques infected with SARS-CoV-2. *bioRxiv* 2020. <https://doi.org/10.1101/2020.03.13.990226> [Online Ahead of Print].
- [8] Farnsworth CW, Anderson NW. SARS-CoV-2 serology: much hype, little data. *Clin Chem* 2020. <https://doi.org/10.1093/clinchem/hvaa107> [Online Ahead of Print].