Clin. Lab. 2021;67:XXX-XXX

SHORT COMMUNICATION

Longitudinal Study of the Signal Strength in two Serologic Assays for Detection of SARS-CoV-2 Specific Antibodies

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SUMMARY

Background: **In the course of the current SARS-CoV-2 pandemic, antibody assays provide an important means for guidance of public health efforts. Thus, characterization of the course of antibody signals on different widely used assays is needed.**
Methods: **We selected 25 PCR-confirmed SARS-CoV-2 cases among 3,273 healthcare workers and measured the course of the antibody signal using the Abbott Architect SARS-CoV-2 IgG assay and the Roche Elecsys® Anti-SARS-CoV-2 immunoassay. The signal strength was then modelled using linear mixed models adjusted for age.**
Results: **Since first sampling, the assay signal decreased per day in the Abbott assay (standardized slope (β) = -0.46, 95% CI = -0.54 to -0.39). In contrast, an increase in the signal was ascertained by the Roche immunoassay per day (β = 0.25, 95% CI = 0.09 to 0.41).**
Conclusions: **Roche Elecsys® Anti-SARS-CoV-2 immunoassay may exhibit greater sensitivity in detecting SARS-CoV-2-specific antibodies in individuals in late stages of postinfection.**
(Clin. Lab. 2021;67:xx-xx. DOI: 10.7754/Clin.Lab.2021.210441)

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Short Communication accepted April 29, 2021

KEY WORDS

COVID-19, molecular diagnostics, blood bank & transfusion medicine

INTRODUCTION

In 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged as a major human pathogen in China [1,2]. SARS-CoV-2 is the etiologic agent of coronavirus disease-2019 (COVID-19), which ranges from asymptomatic to severe, often fatal, lower respiratory tract infections that require intensive care [3]. COVID-19 is routinely diagnosed using real-time RT-PCR to detect viral material in samples that persist in the upper respiratory tract for up to 2 weeks in severe cases. The efficacy of COVID-19 diagnosis by RT-PCR is limited, however, by the frequency of false negatives,

which differs significantly dependent upon time post-exposure [4-6]. False negatives represent ~20% of the results obtained on day 3 following the onset of symptoms and increase to > 66% by day 21 and thereafter [6]. Serological testing offers a means of identifying RT-PCR-negative individuals who experience asymptomatic infections, as well as symptomatic patients who no longer shed virus.

SARS-CoV-2-specific antibodies appear during the course of infection after detection via RT-PCR abates. Median seroconversion time is 11 - 14 days [7,8]. Antibodies are present in < 40% of patients during the first 7 days of illness, but rapidly increase thereafter reaching nearly 100% by day 19 [9]. Consequently, serological testing offers limited benefit in diagnosing acute SARS-Cov-2 infections. Rather, serological testing promises to provide a detailed assessment of the prevalence SARS-CoV-2 in a population [10]. In addition, the results of testing can improve the diagnostic sensitivity of SARS-CoV-2 infection determined by RT-PCR testing alone, determine the therapeutic value of convalescent serum, establish a causal relationship to multisystem inflammatory syndrome in children, and evaluate the potential efficacy of vaccines [11,12]. It should be noted that serology is unable to differentiate current, ongoing infections from those already resolved.

The sensitivity and specificity of the antibody assays during the early phase of SARS-CoV-2 infection have been the focus of most studies conducted to date. Definitive data relevant to postinfection immunity and long-term SARS-CoV-2-specific antibody production are lacking. The following study was undertaken to ascertain antibody production by SARS-CoV-2-positive patients over an approximate 3-month period following initial diagnosis. The Abbott Architect SARS-CoV-2 IgG Assay, an automated two-step immunoassay using chemiluminescent microparticle technology (Abbott Laboratories, Abbott Park, IL, USA), was used and compared to the Roche Elecsys® Anti-SARS-CoV-2 assay, an electro-chemiluminescence immunoassay (Roche Diagnostics GmbH - Mannheim, Germany) that quantifies total SARS-CoV-2-specific immunoglobulin. Both assays are characterized by high rates of sensitivity and specificity, 100% and > 99.6%, respectively [13].

MATERIALS AND METHODS

Participant samples

Serum samples were obtained from 3,273 individuals employed at the University Medical Center of the Johannes Gutenberg-University Mainz in April, May, and July of 2020. SARS-CoV-2-infected participants were identified by RT-PCR screening in the context of another study. The presence of SARS-CoV-2-specific antibody in sera collected initially from these individuals (0 time) and subsequently collected after intervals of 2- to 3-weeks and ~3-months was determined using the Abbott and Roche SARS-CoV-2 immunoassays (Table 1).

Statistical Analysis

To account for the lack of information on infection onset and, thus a defined starting point for the onset of antibody production, the course of the antibody-signal was modelled using linear mixed models from the R package lme4 [14] using random intercepts for each patient, stratified by immunoassay. All models were adjusted for age. Additional adjustment for gender did not improve the models with regard to Akaike's information criterion.

RESULTS

Twenty-five participants who tested SARS-CoV-2 antibody positive were included in this study (Table 1). The data document the stronger signals (signal/cutoff, S/CO) of the Roche Elecsys® Anti-SARS-CoV-2 immunoassay, which generally increased with time (Figure 1). Conversely, the sample-signal/cutoff (S/CO) values of the Abbott SARS-CoV-2 IgG assay decreased over the 3-month period; indeed, 2 samples were negative (1.4 cutoff) at completion of the study.

An overall decrease per day [standardized slope (β) = -0.46, 95% CI = -0.54 to -0.39] in the S/CO ratio since 0 time was determined using the Abbott SARS-CoV-2 IgG assay. In sharp contrast, an increase in the S/CO ratio per day (β = 0.25, 95% CI = 0.09 to 0.41) was ascertained by the Roche Elecsys® Anti-SARS-CoV-2 immunoassay.

Box-plots confirm a marked decrease in the median S/CO ratios determined by the Abbott SARS-CoV-2 IgG assay over time (Figure 2). This decrease was most dramatic between the second and third measurements (i.e., between 2- to 3-weeks and ~3-months). Little change in the median ratios was observed in the results of assays conducted between 0 time and 2- to 3-weeks. In marked contrast, there was an overall increase in the median signal/cutoff ratios determined by the Roche Elecsys® Anti-SARS-CoV-2 immunoassay over the 3-month period; the biggest increase occurred between the initial assay at 0 time and 2- to 3-weeks.

DISCUSSION

During the course of any infection, specific antibody concentrations eventually drop to a minimal level of detection. Consequently, the sensitivity of the serologic assay used for antibody detection is particularly important at later time points. Roche Diagnostics and Abbott Laboratories are leading manufacturers of medical devices and health care products. Both the Roche Elecsys® Anti-SARS-CoV-2 immunoassay and Abbott's Architect SARS-CoV-2 IgG assay have US Federal Drug Administration Emergency Use Approval and the EU CE mark to assess the presence of SARS-CoV-2-specific antibody in serum samples.

The results of the study reported herein document the

Table 1. SARS-CoV-2-specific antibodies in sera collected from 25 COVID-19, RT-PCR-positive study participants were determined by the assay as indicated [a].

Participant	Age	Abbott [b]	Roche [b]	Days [c]	Abbott	Roche	Days [c]	Abbott	Roche
1	26	8.75	99.50	20	8.67	108.70	97	6.12	159.70
2	44	8.12	84.89	20	7.94	124.70	76	5.35	107.30
3	29	5.26	15.40	22	4.84	15.40	81	3.28	NA [d]
4	38	2.75	12.79	19	3.77	58.91	96	2.21	94.45
5	27	7.56	20.51	22	7.14	26.52	85	5.49	12.20
6	26	5.56	29.17	20	4.52	23.37	94	1.98	13.89
7	48	6.17	57.59	20	5.05	90.92	90	2.16	55.91
8	48	8.55	89.80	14	8.65	101.90	79	7.59	55.91
9	40	7.59	94.88	21	7.83	111.30	84	6.03	148.40
10	26	4.22	60.69	16	3.53	83.48	86	1.88	71.60
11	48	7.08	29.08	19	7.75	87.32	81	6.02	136.10
12	22	3.87	NA [d]		NA [d]	NA [d]	80	1.04	NA [d]
13	55	8.03	70.91	21	7.79	115.10	84	5.12	145.80
14	33	6.88	106.60	19	6.59	128.70	80	4.87	NA [d]
15	32	6.89	24.66	24	7.22	108.10	93	7.28	122.60
16	24	5.67	NA [d]		NA [d]	NA [d]	76	3.65	NA [d]
17	31	7.28	70.34	21	7.46	102.10	97	5.13	132.80
18	25	6.34	36.58	20	5.12	67.14	90	2.42	64.84
19	33	6.94	67.22	20	5.40	56.24	89	1.67	21.69
20	44	8.22	70.02	21	7.86	104.40	83	5.46	127.80
21	32	1.79	NA [d]		NA [d]	NA [d]	99	0.91	NA [d]
22	56	3.49	15.34	21	2.85	39.70	103	1.50	43.22
23	24	3.92	19.93	20	3.67	55.58	96	1.80	37.48
24	27	7.02	102.80	14	6.75	125.50	81	4.66	NA [d]
25	45	4.72	35.34	20	3.68	56.48	83	1.81	40.10

[a] Data are signal/cutoff ratios.
[b] Values for the first sample collection (0 time).
[c] Lapsed time since the first sample collection.
[d] Not available.

general increase in signal strength of the Roche assay over the first 3 months postinfection; the Abbott assay signal, on the other hand, decreased during the same time period. These results are in general agreement with those of Tan and co-workers who reported a moderate correlation between the Roche and Abbott assays [15]. They found, however, that the Roche assay generated greater signal intensities and was more sensitive when used to detect antibodies in samples obtained from COVID-19 patients at 14 - 20 days and > 21 days after symptom presentation [15]. Both the Roche and Abbott assays rely on the detection of antibodies specific for recombinant, SARS-CoV-2 nucleocapsid antigen. A key difference resides in the ability of the Roche Elecsys® Anti-SARS-CoV-2 immunoassay to assess total antibody production while Abbott's Architect SARS-CoV-2 IgG assay is IgG specific.

The current study suffers from several limitations: relatively small sample size (n = 25), population variation including some incomplete cases, and lack of a defined starting point (i.e., 0 time). Moreover, the simpler linear regression model chosen here for the sake of interpretability may be limited in flexibility compared to splines or polynomial models.

It is important to note that there is currently no evidence to support the fact that people who recover from COVID-19 and possess antibodies are immune and protected from SARS-CoV-2 re-infections. The correlation between SARS-CoV-2-specific antibody production and immunity remains to be firmly established [12]. Neutralizing antibody titers are highly variable, non-detectable in some patients yet persistent in others [11,16,17].

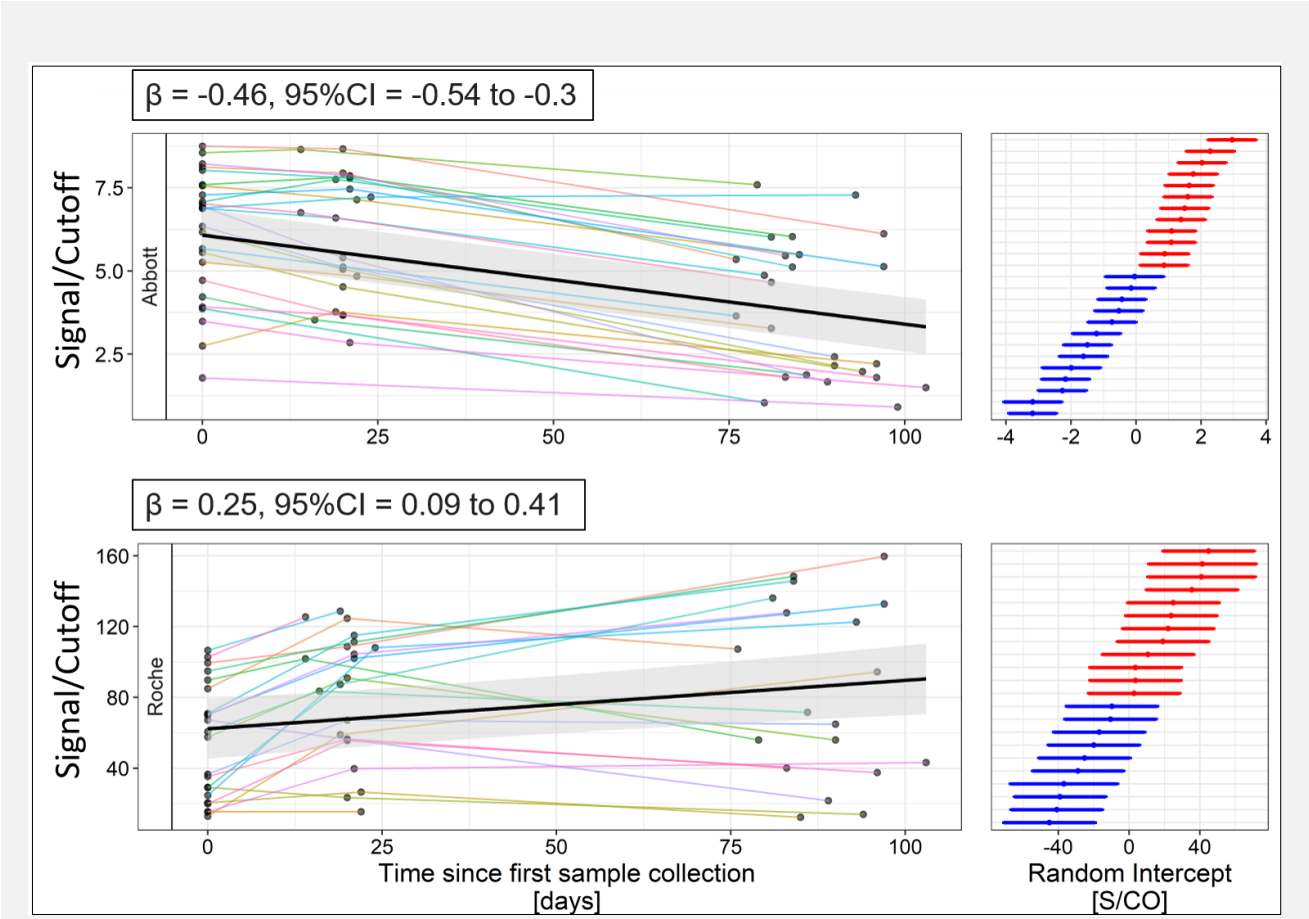

Figure 1. Scatterplot comparing signal/cutoff ratios versus time since first sample collection obtained for the Abbott (top) and Roche (bottom) assays.

Black lines represent S/CO ratios predicted by linear mixed-model regression analysis (fixed effects: days since 0 time and age; random effects: random intercept per participant). Surrounding grey bands indicate 95% confidence intervals. Random intercepts per participant are displayed in the panels on the right.

As such, the Infectious Diseases Society of America strongly advise against associating positive serology with immunity [12]. Nonetheless, characterizing immunity has important implications with respect to transmission modelling, ascertaining population susceptibility, and determining the therapeutic efficacies of vaccines and convalescent plasma.

CONCLUSION

The Roche Elecsys® anti-SARS-CoV-2 assay exhibits greater sensitivity than Abbott Laboratories' SARS-CoV-2 IgG assay in detecting SARS-CoV-2-specific antibody in serum samples derived from COVID-positive individuals late postinfection. Whether this is due to the ability of the Roche assay to detect total (IgA, IgG, and IgM class) antibody or the lower sensitivity of the Abbott assay remains to be determined.

Acknowledgment:
The authors thank Dr. Stephen H. Gregory (Providence, RI, USA) for his help writing and editing this manuscript.

Data Availability:
All data relevant to the study are included in the article.

Source of Funds:
This study received no financial support from a specific funding agency.

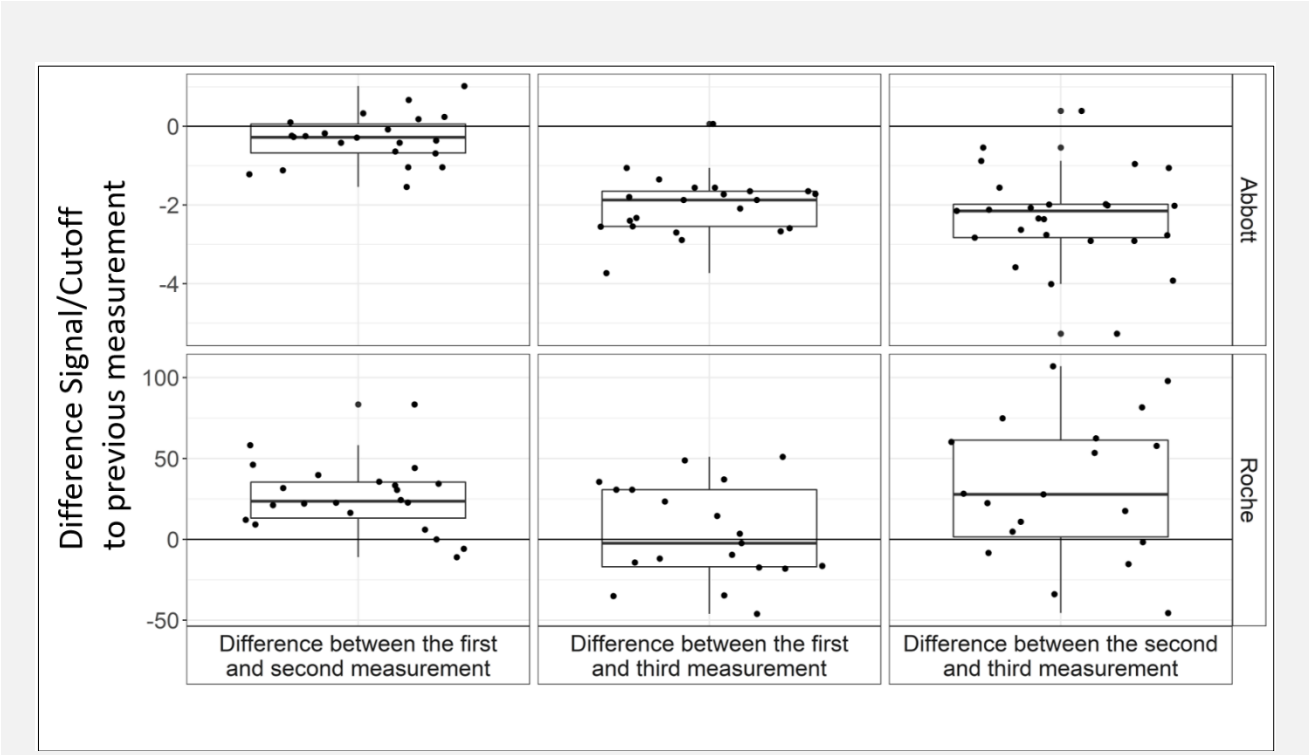

Figure 2. Boxplots showing differences in signal/cutoff ratios between Abbott (top) and Roche (bottom) measurements at: 0 time and 2- to 3-weeks, 0 time and 3-months, and 2- to 3-weeks and 3-months.

Declaration of Interest:

None to declare.

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