Longitudinal Assessment of SARS-CoV-2 Anti-Nucleocapsid and anti-Spike-1-RBD Antibody Testing Following PCR-Detected SARS-CoV-2 Infection

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ABSTRACT

Background*:* SARS-CoV-2 serologic assays are becoming increasingly available and may serve as a diagnostic aid in a multitude of settings relating to past infection status. However, there is limited literature detailing the longitudinal performance of EUA-cleared serologic assays in US populations, particularly in cohorts with a remote history of PCR-confirmed SARS-CoV-2 infection (e.g., > 2 months).

Methods*:* We evaluated the diagnostic sensitivities and specificities of the Elecsys® Anti-SARS-CoV-2 (anti-N) and Elecsys® Anti-SARS-CoV-2 S (anti-S1-RBD) assays, using 174 residual clinical samples up to 267 days post-PCR diagnosis of SARS-CoV-2 infection (n=154) and a subset of samples obtained prior to the COVID-19 pandemic as negative controls (n=20).

Results*:* The calculated diagnostic sensitivities for the anti-N and anti-S1-RBD assays were 89% and 93%, respectively. Of the 154 samples in the SARS-CoV-2-positive cohort, there were 6 discrepant results between the anti-N and anti-S1-RBD assays, 5 of which were specimens collected ≥ 200 days post-PCR positivity and only had detectable levels of anti-S1-RBD antibodies. When only considering specimens collected ≥ 100 days post-PCR positivity (n=41), the sensitivities for the anti-N and anti-S1-RBD assays were 85% and 98%, respectively.

Conclusions*:* The anti-S1-RBD assay demonstrated superior sensitivity at time points more remote to the PCR detection date, with 6 more specimens from the SARS-CoV-2-positive cohort detected, 5 of which were collected more than 200 days post-PCR positivity. While analytical differences and reagent lot-to-lot variability are possible, this may indicate that, in some instances, anti-S1-RBD antibodies may persist longer *in vivo* and may be a better target for detecting remote SARS-CoV-2 infection.

IMPACT STATEMENT

In this focused report, we characterize the longitudinal performance of two clinical serologic assays, detecting anti-N and anti-S1-RBD antibodies, using 154 residual clinical samples up to 267 days post-PCR diagnosis of SARS-CoV-2 infection and a subset of samples obtained prior to the COVID-19 pandemic as negative controls. We found that the assays evaluated in this report are highly sensitive and specific and remain positive in most specimens up to 267 days post-PCR detection of SARS-CoV-2 infection. Lastly, the Elecsys® Anti-SARS-CoV-2 S (anti-S1-RBD) assay was found to be slightly more sensitive when analyzing specimens drawn ≥ 100 days post-PCR confirmation.

INTRODUCTION

In the ongoing COVID-19 pandemic, molecular-based diagnostics remain the gold standard for detecting acute infection (1). Serologic assays are becoming increasingly available and may serve as a diagnostic aid in a multitude of settings relating to past infection status. The clinical utility of these tests remains part of an ongoing debate, but proposed indications include seroprevalence studies, the detection of asymptomatic infections, vaccine responsiveness, and cases where clinical suspicion of past or present SARS-CoV-2 infection is high, but PCR is negative (2,3). Currently, there is limited literature detailing the performance of emergency use authorization (EUA)-cleared serologic assays in US populations, particularly in cohorts with a remote history of PCR-confirmed SARS-CoV-2 infection (e.g., ≥ 2 months) (4–7). As a result, interpretation of these assays can be challenging when there is limited data to advise clinicians as to how long antibody tests can be expected to be positive in the general population. Accordingly, there remains a significant need for additional investigation with respect to this, and the longitudinal assessment of antibody assay performance metrics.

Among the currently available EUA-cleared assays, there are a multitude of method formats which measure different antibody isotypes and target a variety of SARS-CoV-2 epitopes (4). Recently, two assays have been cleared through the EUA pathway which offer; (1) the qualitative measurement of high affinity total antibodies directed against the nucleocapsid (N) antigen (Elecsys® Anti-SARS-CoV-2) (Roche Diagnostics; Rotkreuz, Switzerland) and (2) the semi-quantitative measurement of high affinity total antibodies directed against receptor binding domain (RBD) portion of the spike (S) protein on the S1 subunit (Elecsys® Anti-SARS-CoV-2 S) (Roche Diagnostics; Rotkreuz, Switzerland). To our knowledge, and at the time of this writing, there are only two publications that investigate the persistence of anti-S1-RBD antibodies at time points ≥ 2-3 months post-PCR positivity (4,5). In this focused report, we present data from our internal clinical validation experiments which sought to characterize the

longitudinal performance of two commercially available, EUA-cleared assays, targeting anti-S1-RBD and anti-N antibodies, up to 267 days post-PCR diagnosis of SARS-CoV-2 infection.

METHODS:

This study was reviewed and approved by the Yale University Internal Review Board as an exempt study using deidentified clinical excess specimens (IRB#2000028760). De-identified residual patient plasma samples were retrospectively identified for this project using previously described methods (8–10). Residual plasma samples were obtained from two cohorts: (1) SARS-CoV-2-positive and (2) SARS-CoV-2-negative. SARS-CoV-2-positive samples included those with a history of SARS-CoV-2 infection, as defined by a positive SARS-CoV-2 PCR test documented in our electronic health record (EHR), which includes external laboratory results. Samples included in the SARS-CoV-2-positive cohort were also annotated with the number of days from the date of first SARS-CoV-2 PCR-positivity to the date of sample collection. Residual plasma samples in the SARS-CoV-2-positive cohort were obtained from light green-top (LGT)(lithium heparin) containers (Becton Dickinson; Franklin Lakes, NJ) which were originally collected as part of routine clinical workflow. On receipt in the laboratory, LGT specimens were accessioned, immediately centrifuged following, and ordered tests were run off the primary specimen container. Following completion of testing, LGT containers were temporarily stored in a temperature-controlled output buffer for 6 hours and were subsequently transferred to a refrigerator (2-8^{o}C) for short term storage (5-7 days) until discard. Prior to discard, these LGT containers were identified (10), plasma was aliquoted, and refrigerated (2-8^{o}C).

SARS-CoV-2-negative samples were collected prior to the first positive SARS-CoV-2 PCR test at our institution (March 2020). Residual plasma samples from the SARS-CoV-2-negative cohort were originally collected for amino acid profile testing. These samples were collected in dark green-top (sodium heparin) containers (Becton Dickinson; Franklin Lakes, NJ). As part of routine clinical workflow,

these specimens were transported to the laboratory on ice and upon receipt, immediately aliquoted and stored in a freezer (-20^{o}C). Samples were subsequently thawed for amino acid profile testing and then refrozen. Accordingly, the SARS-CoV-2-negative plasma samples included for this project were likely subjected to two freeze-thaw cycles.

Aliquoted plasma specimens were analyzed using the Elecsys® Anti-SARS-CoV-2 and the Elecsys® Anti-SARS-CoV-2 S, which measure anti-N and anti-S1-RBD total antibodies, respectively. Both assays use a double-antigen sandwich (DAGS) electrochemiluminescence format. The anti-S1-RBD assay is a semi-quantitative assay which has an analytic measurement range (AMR) of 0.40-250 U/mL, and a clinical reportable range (CRR) up to 2500 U/mL using an on-board 1:10 dilution. A concentration ≥ 0.80 U/mL is considered 'reactive' whereas < 0.80 U/mL is considered 'non-reactive'. The anti-N assay is a qualitative assay wherein a cut-off index ≥ 1 is considered 'reactive'. All serologic testing was performed on a COBAS e801 (Roche Diagnostics; Risch-Rotkreuz, Switzerland) and in a manner that is consistent with the manufacturer's instructions and recommended cutoffs. Both assays were also validated with respect to method correlation, linearity (for anti-S1-RBD), reference range verification, freeze-thaw stability over two freeze-thaw cycles, inter-precision, and intra-precision (data not shown). Lastly, a Fisher's exact test was used to calculate confidence intervals for specificity and sensitivity.

RESULTS:

A total of 174 residual plasma specimens were included in the final analysis, 20 of which were from the SARS-CoV-2-negative cohort and 154 from the SARS-CoV-2-positive cohort. The SARS-CoV-2-negative specimen collection dates ranged from December 2018 to February 2020. SARS-CoV-2-positive specimen collection dates ranged from November 2020 to January 2021, with a mean patient age of 64.7 (SD 17.1) years and a mean of 79 (IQR: 21-121, Range: 14-267) days between the first positive SARS-CoV-2 PCR result and LGT collection.

Both serologic assays were non-reactive for all specimens (n=20) from the SARS-CoV-2-negative cohort with a calculated diagnostic specificity of 100% (95% CI 84-100%). In the SARS-CoV-2-positive cohort, 17 specimens were non-reactive for anti-N antibodies, yielding a diagnostic sensitivity of 89% (95% CI 82-93%) (Figure 1A). The anti-S1-RBD assay was non-reactive for a total of 11 specimens in the SARS-CoV-2-positive cohort, yielding a diagnostic sensitivity of 93% (95% CI 88-96%) (Figure 1B). Of the total 154 samples in the SARS-CoV-2-positive cohort, there were 6 discrepant results between the anti-N and anti-S1-RBD assays, 5 of which included specimens collected ≥ 200 days post-PCR positivity and only had detectable levels of anti-S1-RBD antibodies. Both the anti-N and anti-S1-RBD assays demonstrated positivity across the entire observation period, with detection signal at or near the upper limit of quantitation for both methods beyond 200 days post-PCR positivity (Figure 2A and 2B).

DISCUSSION:

While there are several publications detailing serologic kinetics and test performance during the initial 2-4 weeks following SARS-CoV-2 infection, there is little published data which describe assay performance metrics ≥ 2-3 months after infection (11,12). In this focused report, we describe the longitudinal performance characteristics of two automated serology assays up to 267 days post-PCR positivity. Throughout the post-PCR positivity specimen collection date range, the anti-S1-RBD assay demonstrated superior sensitivity, detecting 6 more specimens in the SARS-CoV-2-positive cohort relative to the anti-N assay, 5 of which were collected more than 200 days post-PCR positivity. When, only considering specimens collected ≥ 100 days post-PCR positivity (n=41), the calculated sensitivity for the anti-N and anti-S1-RBD assays was 85% (95% CI 71-93%) and 98% (95% CI 87-100%), respectively. Both assays evaluated in this report use the same antibody detection format (DAGS) and share the same manufacturer. While analytical differences and reagent lot-to-lot variability are possible, our findings

may indicate that, in some instances, anti-S1-RBD antibodies may persist and be detectable longer *in vivo* and may be a better target for identifying remote SARS-CoV-2 infection.

Previously published literature has shown that, in most patients, the initial serologic response following SARS-CoV-2 infection will occur within two weeks post-symptom onset (3,12,13). Of note, the claimed sensitivities by the manufacturer for the anti-N and anti-S1-RBD assays for specimens collected ≥ 14-15 days post-PCR confirmation were 99.5 and 96.6%, respectively. During this timeframe post-infection, recent peer-reviewed literature described observed sensitivities for the Elecsys® Anti-SARS-CoV-2 (anti-N) and the Elecsys® Anti-SARS-CoV-2 S (anti-S1-RBD) assays to be around 90% (13,14). This is consistent with our findings, wherein our calculated sensitivity for anti-N and anti-S1-RBD between 2- and 3-weeks post-PCR positivity (n=44) was 86 and 89%, respectively. Discrepancies between performance metrics reported in the literature and by the manufacturer are likely due to differences in tested populations regarding characteristics such as disease severity and host immune status. As shown by Schaffner et al., there are a multitude of clinical characteristics which can negatively influence post-infection serologic response, as determined by the Elecsys® Anti-SARS-CoV-2 S assay. These include disease severity, smoking status, and the presence or absence of a fever (4). Accordingly, it is generally accepted that negative serologic testing should not be used to rule out a history of past infection.

One limitation of the present study is the case definition chosen for SARS-CoV-2 infection. While the date of SARS-CoV-2 PCR positivity is a reliable and easily accessible indicator of infection, it does not necessarily correlate perfectly with the date of symptom onset and, despite a high specificity, when used in the setting of broad screening, can potentially identify large numbers of false positive cases. Accordingly, these discrepancies can yield differences between observed and expected serologic testing results. An additional limitation is the small sample size in the SARS-CoV-2-negative cohort (n=20) which limits the generalizability of the specificity results.

Overall, both the Elecsys® Anti-SARS-CoV-2 (anti-N) and Elecsys® Anti-SARS-CoV-2 S (anti-S1-RBD) assays demonstrated good sensitivity throughout the observation period. The Elecsys® Anti-SARS-CoV-2 S (anti-S1-RBD) assay was found to be slightly more sensitive when analyzing specimens drawn ≥ 100 days post-PCR confirmation, although the differences observed were not statistically significant. These data are useful in guiding the clinical interpretation of serologic testing in patients who may have a remote history of SARS-CoV-2 infection.

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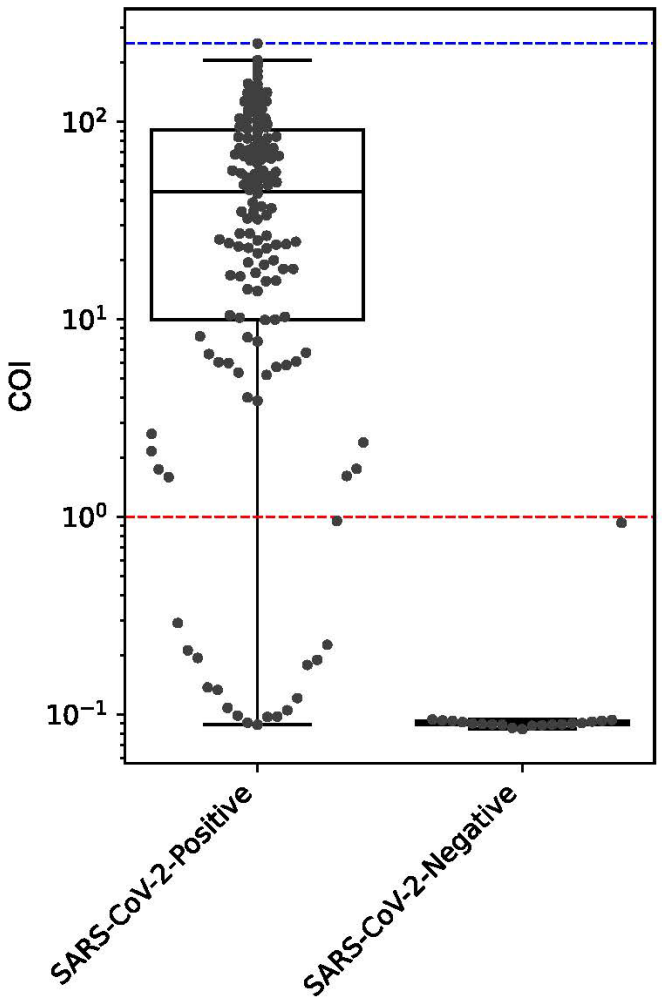
FIGURES:

Figure 1: Distribution of quantitative signal (y-axis, log scale) for anti-N and anti-S1-RBD antibody assays in the SARS-CoV-2-positive and SARS-CoV-2-negative cohorts. (A) Total Ig Anti-N (Elecsys® Anti-SARS-CoV-2; Roche Diagnostics; Risch-Rotkreuz, Switzerland). Red dashed line represents the positivity threshold (≥ 1.0 COI) and the blue dashed line represents the upper limit of quantitation (250 COI). (B) Total Ig anti-S1-RBD (Elecsys® Anti-SARS-CoV-2 S; Roche Diagnostics; Risch-Rotkreuz, Switzerland). Red dashed line represents the positivity threshold (≥ 0.8 U/mL) and the blue dashed line represents the upper limit of quantitation (2500 U/mL).



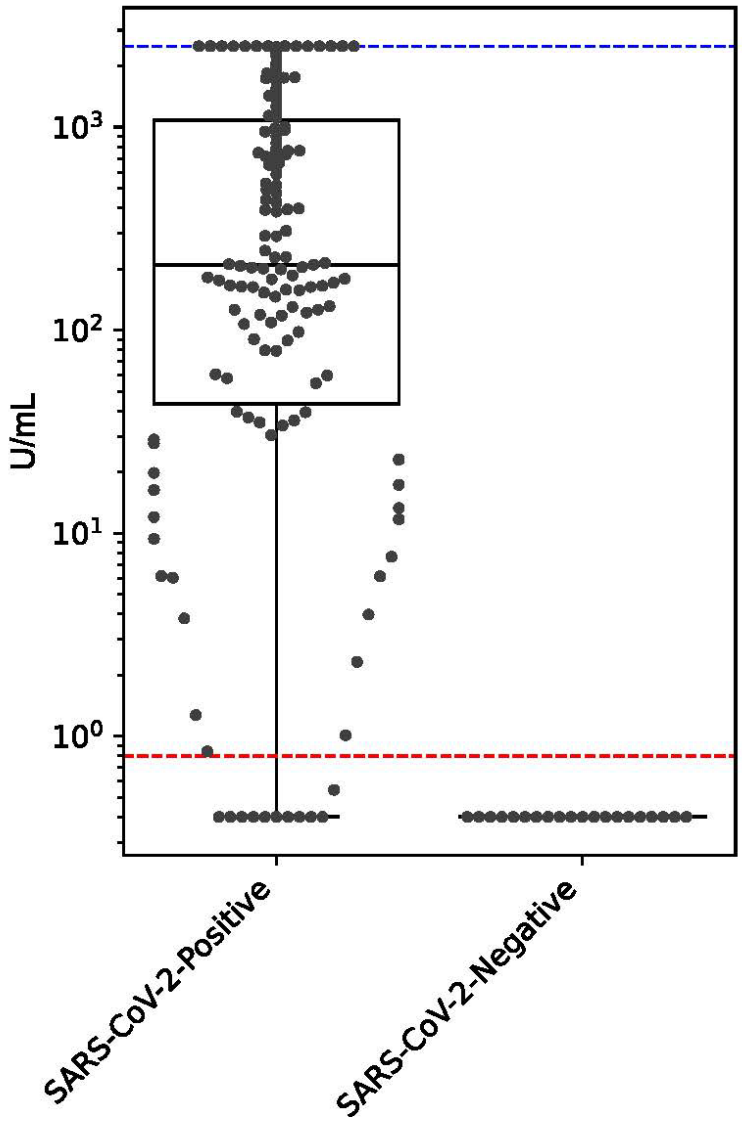

10^3
10^2
10^1
10^0
U/mL
SARS-CoV-2-Positive
SARS-CoV-2-Negative

Figure 2: Distribution of quantitative signal (y-axis, log scale) for anti-N and anti-S1-RBD antibody assays plotted as a function of days post-SARS-CoV-2 PCR positivity. (A) Total Ig Anti-N (Elecsys® Anti-SARS-CoV-2; Roche Diagnostics; Risch-Rotkreuz, Switzerland). Red dashed line represents the positivity threshold (≥ 1.0 COI) and the blue dashed line represents the upper limit of quantitation (250 COI). (B) Total Ig anti-S1-RBD (Elecsys® Anti-SARS-CoV-2 S; Roche Diagnostics; Risch-Rotkreuz, Switzerland). Red dashed line represents the positivity threshold (≥ 0.8 U/mL) and the blue dashed line represents the upper limit of quantitation (2500 U/mL).