

## Letter to the Editor

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# Prospective serological evaluation of anti SARS-CoV-2 IgG and anti S1-RBD antibodies in a community outbreak

<https://doi.org/10.1515/cclm-2021-0127>

Received January 28, 2021; accepted February 14, 2021;  
published online February 23, 2021

**Keywords:** anti S1-RBD Ab; COVID-19; immunoglobulin IgG; SARS-CoV-2.

To the Editor,

There is a significant interest in the potential role for the serological tests [1], which predominantly concerns the immunoglobulin (Ig)G, characterized by a more prolonged response with respect to IgM and IgA. The information relative to the dynamic response of IgG is not completely clear yet, especially for what concerns their persistence as well as the possibility to quantify the IgG with different antigenic target.

Most assays target either the spike glycoprotein (S) or the nucleocapsid-protein (N) of SARS-CoV-2, but recently assays target specifically towards receptor binding domain (RBD) of S1 subunit of the SARS-CoV-2 spike protein are available.

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Antibodies to RBD have been shown to correlate with virus neutralization [2].

This study involved all the guests of a long-term care facility (LTCF) where an SARS-CoV-2 outbreak developed in late March 2020. All guests were studied for the Coronavirus infection by oro/nasopharyngeal swab using a real time polymerase chain reaction method (rRT-PCR, Cobas 6800, Roche Diagnostics GmbH, Mannheim, Germany) within a period stretching from March 29 to April 22, 2020.

After first swab, all the subjects were monitored with subsequent swabs after 20 days, 30 days (28–36) and only if still positive cases, after 50 days. At the same time, during the second and third control, the residents were investigated for IgG anti SARS-CoV-2 antibodies, using a quantitative chemiluminescent method Maglumi 2019 CoV IgG. Cut-off is 1.1 AU/mL with a grey zone between 0.9 and 1.1 AU/mL for IgG.

The guest underwent further checks after 120 and 180 days from the detection of positivity for SARS-CoV-2 infection.

These samples were investigated for IgG antibodies anti SARS-CoV-2 but also for detecting antibodies addressed against the spike surface protein (S) and the spike RBD.

We evaluate three different commercial immunoassays:

- (1) Maglumi® SARS-CoV-2 S-RBD IgG (CLIA)(Shenzen New Industries Biomedical Engineering Co, Snibe diagnostics, Shenzen, PR China) (SNIBE-IgG-RBD), a method for quantitative determination of S-RBD IgG antibodies to SARS-CoV-2. Positive cut-off is  $\geq 1.0$  AU/mL.
- (2) Elecsys® anti SARS-CoV-2 S (Roche Diagnostic GmbH, Mannheim, Germany) implemented on Cobas 411, an immunoassay for the *in vitro* quantitative determination of antibodies to the SARS-CoV-2 spike (S) protein RBD in human serum and plasma. Cut-off is  $\geq 0.8$  U/mL.

(3) Atellica IM<sup>®</sup> SARS-CoV-2 IgG (sCOV2G) (Siemens Healthineers, Tarrytown, NY, USA) (SIEMENS-IgG-RBD) a quantitative method for detection IgG antibodies against S1-RBD antigen. Cut-off is  $\geq 1.0$  Index.

The study was approved by the Ethics Committee and was done in compliance with the World Medical Association Declaration of Helsinki.

Among 65 guests residing in the LTCF, 54 resulted positive for SARS-CoV-2 on the swab but 11 rapidly (within one month) died. Eleven subjects did not develop the infection (permanently negative swabs until now). Monitoring therefore involved 43 guests positive for SARS-CoV-2 infection at swab (eight males, 35 females, average age 88 years [56–97]) for two months, but other two died after three months and one returned home. The follow up was hence complete in 40 guests.

During the first monitoring period of the positive patients, IgG antibodies against nucleocapsid-protein (SNIBE 2019-nCoV IgG) raised over the positive cut-off (1.1 AU/mL) in 39 of 43 patients (90%), in line with other findings [3].

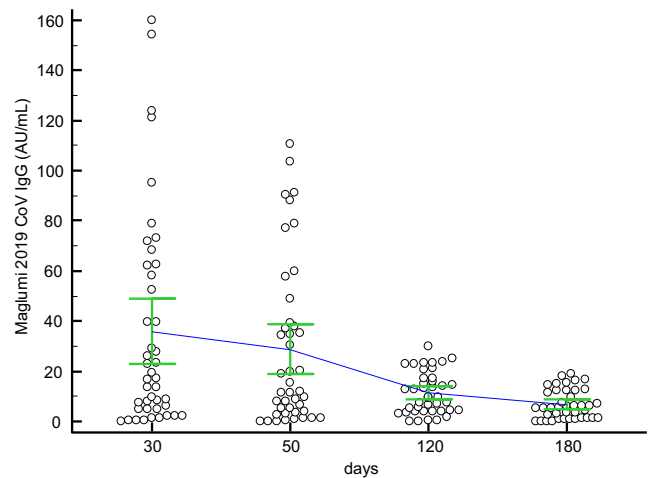
The four patients with IgG-Ab anti SARS-CoV-2 values below the cut-off remained negative even in subsequent controls (after 50, 120 and 180 days). Those patients resulted asymptomatic on the basis of the evaluation of some key parameters: asthenia, fever, oxygen saturation and need for oxygen therapy. Other studies underlined how the immune response among asymptomatic patients may be weaker [4].

The IgG-Ab anti SARS-CoV-2 median concentration observed in the first control was 11.7 AU/mL in asymptomatic patients (n. 20) and 23.1 AU/mL in symptomatic patients. The difference does not reach statistical significance, perhaps due to the meagreness of the sample. However, seven patients with high humoral response ( $>70.0$  AU/mL) were all symptomatic.

Among the patients who completed the follow up (n. 40), all those who had IgG-Ab anti SARS-CoV-2 titer above the cut-off in the first control (n. 36) had a value above the cut-off also in the subsequent measurements, 50, 120 and 180 days after diagnosis. However, the IgG-Ab anti SARS-CoV-2 value drop out in a statistically significant way after each check 17.0 vs. 12.1 AU/mL  $p < 0.001$  (n. 43); 12.1 vs. 9.5  $p < 0.0001$  (n. 41); 9.4 vs. 5.5  $p < 0.0001$  (n. 40) (Wilcoxon test) (Figure 1).

Antibodies directed against the RBD domain of the S1 protein SARS-CoV-2, were also measured, during the controls after four and six months, by using three assays from different companies.

The concentration of ROCHE RBD antibodies anti SARS-CoV-2 is above the cut-off in all patients, both after



**Figure 1:** Comparison of Ab concentrations measured by Maglumi 2019 CoV IgG (Snibe) in the four control times.

four and after six months. Wilcoxon's paired test shows a non-significant difference between the two controls (median 91.9 vs. 86.6,  $p = ns$ ).

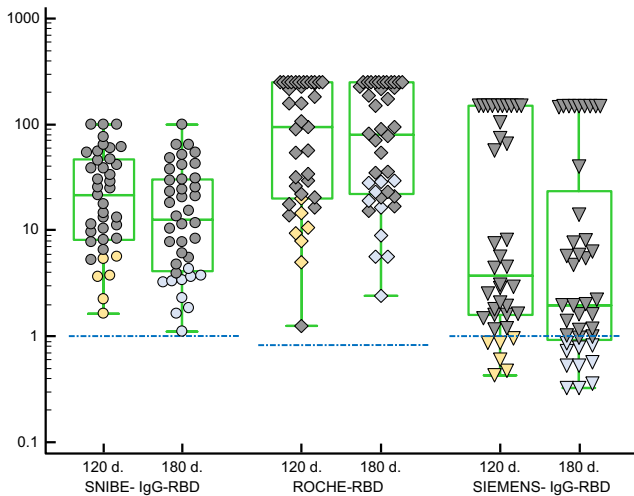
On the contrary, the comparison between the antibodies SNIBE-IgG-RBD shows a statistically significant difference ( $p < 0.001$ ) between the control after 120 days and after 180 days (median 19.6 vs. 12.6), but antibodies higher than the cut-off were found in all patients.

Finally, with the SIEMENS-IgG-RBD assay, six out of 40 patients (20%) were "negative" in the check at time 120 day, having IgG anti-RBD concentration less than 1.0 index, and four more subjects became negative after 180 days. However, only in two cases at time 120 day and two more at 180 day showed an antibody concentration lower than 0.5 index, all the other having detectable IgG concentration.

Even if the compared methods have different units of measurements and scales, the data obtained with the methods of the different companies correlate with each other, the samples that resulted negative with the Siemens method after 120 days from contagion are those showing low antibody titers also with the other methods (Figure 2).

Combining all the data from both the two controls we obtained the following correlations: Ab IgG RBD Snibe vs. Roche:  $\log(y) = 0.557 + 1.059 \log(x)$ ,  $r = 0.89$ ; Ab IgG RBD Snibe vs. Siemens:  $\log(y) = -1.358 + 1.379 \log(x)$ ,  $r = 0.97$ ; Ab IgG Roche vs. Snibe  $\log(y) = 1.615 + 0.734 \log(x)$ ,  $r = 0.88$ .

It is necessary to consider that the methods present different measurement units and very different scales between them. It is thus possible that a better harmonization of the units and the cut-off further improves not only the quantitative but also the diagnostic concurrence of the methods, as has already been proposed by other authors [5].



**Figure 2:** Multiple comparison between the three methods directed against Ab anti S1-RBD, at the third (120 day) and fourth (180 day) control: Maglumi<sup>®</sup> SARS-CoV-2 S-RBD IgG (Snibe diagnostics); Eleysys<sup>®</sup> anti SARS-CoV-2 S, on COBAS 411 (Roche Diagnostic GmbH); Atellica IM<sup>®</sup> SARS-CoV-2 IgG (sCOV2G) (Siemens Healthineers). Data are represented in logarithmic scale.

The quantification of the anti S1-RBD antibodies of the three methods evaluated in this study has shown to be very similar, and the difference of the Siemens method seems to depend essentially from the positioning of the threshold at 0.5 cut-off index (proposed by the producer in an initial pre-launch phase), for “identifiable” concentrations, would have provided a very similar categorization to the other two methods.

It would hence be useful to define, more than a cut-off or perhaps besides the cut-off, also a different threshold value, an antibody titer, able to define the acquired immunity, but for this it will be necessary to wait for future perspective studies.

Another important element is the evaluation of the decrease of the antibody titer over time.

Other studies have monitored the titer of antibodies anti IgG over time. Long et al. [3] documented the evolution of the IgG antibodies in two groups of patients, symptomatic and asymptomatic. In both groups, the control carried out four weeks after the discharge showed a decrease of the IgG antibodies with the negativity of 40% of asymptomatic patients and of 12% of symptomatic patients. In his recent study of the Icelandic population, Gudbjartsson [6] reports the presence of IgG antibodies in 90% of positive SARS-CoV-2 patients admitted into the hospital and documents the persistence of said antibodies, even if with a slight decrease, even in the last check carried out after four months from the diagnosis both for anti N IgG and for anti-RBD IgG.

Our experience confirmed what others studies found. Our patients also show a decline in the concentration of anti N IgG antibodies, which however remain above the cut-off in all the patients who had tested positive, with the exception of the RBD-IgG antibodies dosed with Siemens method, where 25% of patients became negative after 180 days using the cut-off proposed by the company. The antibody titer is probably higher among the Icelandic patients [6] (although this is difficult to verify, given the arbitrariness of the used measures), since in that study all the subjects were symptomatic, being all hospitalized, while 45% patient of ours were asymptomatic.

Furthermore, the follow-up in that study only lasted 120 days, compared to the 180 days in our study.

The study was carried out on a limited group of subjects, albeit very homogeneous with respect to physiological and environmental characteristics. However, the follow up among the guests of the LTCF allows us to conclude that a percentage between 90 and 100% of the LTCF guests (depending on the methods used) shows a high titer of Ab which albeit with a tendency to diminish still remains high even after 180 days. The methods appear to be well correlated between them and interchangeable for an epidemiological evaluation as well as to evaluate the acquired immunity.

**Research funding:** None declared.

**Author contributions:** All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Competing interests:** Authors state no conflict of interest.

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**Ethical approval:** The study was approved by the Ethics Committee and was done in compliance with the World Medical Association Declaration of Helsinki.

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