

Article

# Two SARS-CoV-2 IgG Immunoassays Comparison and Time-Course Profile of Antibodies Response

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**Abstract:** The role of the immune response to SARS-CoV-2 infection is not yet well known, in particular about the persistence of circulating antibodies. The aim of the study is to compare the results of two automated systems for the determination of IgG antibodies against SARS CoV-2 and to assess the time course of the IgG response after the onset of symptoms for a period longer than that evaluated to date. IgG were measured in 98 specimens of 55 subjects with COVID-19 (time from the onset of symptoms from 3 to 109 days) using the automated tests "Abbott SARS-COV-2 IgG" and the "MAGLUMI 2019-nCoV IgG". The two methods had a concordance of 91.8%, but the quantitative correlation showed very dispersed results. All the specimens resulted positive after 17 days from the onset of the symptoms. However, the median concentrations of IgG, after a rapid increase up to about 20 days, quickly decrease to about 15% of the maximum for Maglumi. The same samples measured by Architect showed a quite constant trend up to 80 day, and then an only moderate decline. The titer of IgG against SARS-CoV-2 in patients exposed to COVID-19 may significantly and rapidly decrease, with a different time-course depending on the method used for the determination

**Keywords:** Immune response; SARS-CoV-2 antibodies response; Covid-19 management; Maglumi; Architect; method comparison

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## 1. Introduction

A new coronavirus (2019-nCoV) has emerged in December 2019 in the region of Wuhan, China, and the related disease (COVID-19) to date represent one of a major health concern in the world. The serological tests for CoV-2 antibodies determination could be useful for supporting the assessment of cases of uncertain identification or with moderate illness, as well as for contact tracing and for epidemiological studies [1]. The latter could in turn be helpful for the correct identification of asymptomatic subjects and for correctly estimating the illness and death rate. To date there are several available SARS-CoV-2 serological tests, different papers have already compared some of these methods, and found acceptable classification concordance although very disperse quantitative results [2-6].

However, up to the present time it is uncertain whether the recovery from COVID-19 provides immunity [7]. A rapid seroconversion was demonstrated, but the persistence of IgG is only known in the short-term period, since studies on immune response to COVID-19 are to date mostly limited to 5-6 weeks after the symptoms' onset [8], and little is known about the longer-term response [9,10].

Aim of this study is to compare two different automated methods for antibodies anti SARS-CoV-2 determination and the evaluation of the antibodies kinetic with both the methods up to about 15 weeks.

## 2. Materials and Methods

We collected samples from patients who presented at the Ospedale dell' Angelo (Mestre, Italy) in March 2020 and were diagnosed as COVID-19 affected according to both clinical and laboratory criteria. Only patients with known date of symptoms' onset were considered. Fifty-five patients were then included in the study (46 males, 9 females, median age 63 years, minimum 28, maximum 89). There were 32 patients with one blood sample, 12 with two withdrawals over the following days, 4 with 3 withdrawals, 5 with 4 withdrawals and 2 with 5 withdrawals, for a total of 98 assessed specimens. The median time from the onset of symptoms was 22 days (minimum 3, maximum 109).

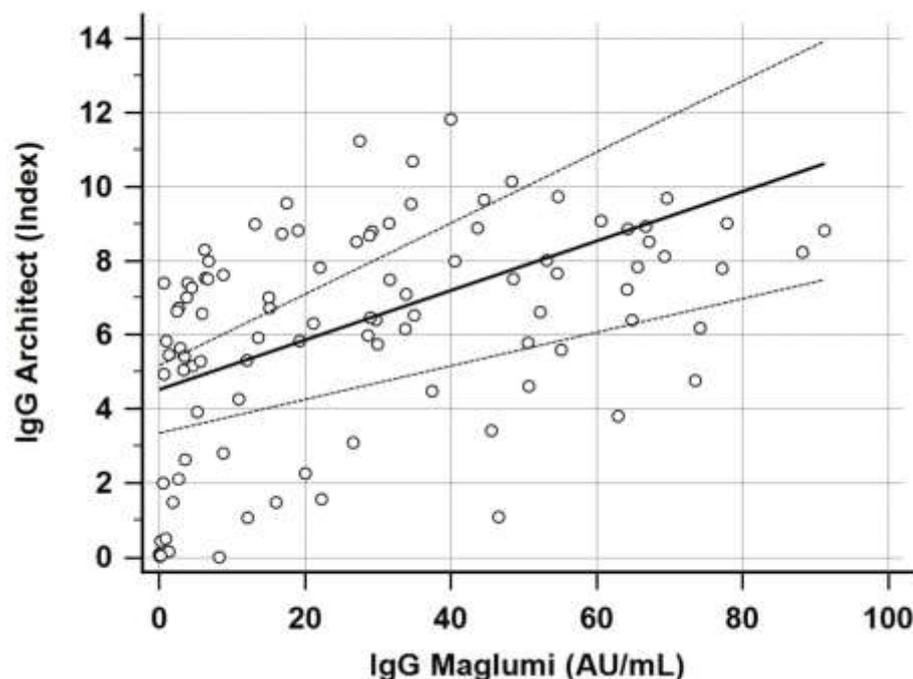
IgG were measured with 2 two-step chemiluminescence microparticle immunoassays, the Abbott SARS-COV-2 IgG (nucleoprotein based antigen) and the MAGLUMI 2019-nCoV IgG (S1, S2 and N proteins based). The good analytical characteristics of the two assays were previously evaluated and confirmed [11-13]. The Abbott SARS-COV-2 IgG assay is calibrated against an internal standard and the results are expressed as Index (ratio between the sample result and the calibrator result). The samples are considered reactive with an index  $>1.4$ . The MAGLUMI 2019-nCoV IgG was calibrated against an internal standard and the results were expressed as Arbitrary Units/mL (AU/mL). The samples are considered reactive with a concentration  $>1.0$  AU/mL.

The assays were carried out on the analyzers Architect I2000sr (Abbott, Illinois, USA) and the Maglumi 800 (Snibe, Shenzhen, China) according to the manufacturer's instructions.

The statistical analysis was performed with MedCalc © Software, Version 19.2.1 (MedCalc Software, Mariakerke, Belgium). All investigations have been conducted by following the tenets of the Declaration of Helsinki and has been complied with institutional policies (Ethical Committee approval n 149/A CESC).

## 3. Results

The qualitative overall concordance between method was 91,8% (kappa statistics, 0.55; 95% CI, 0.28–0.83). The quantitative relationship showed a statistically significant linear correlation (Architect= 0.053 Maglumi + 4.6) with, however, a very disperse distribution of cases. In fact, the Passing-Bablok correlation showed a significant deviation from linearity (Figure 1).



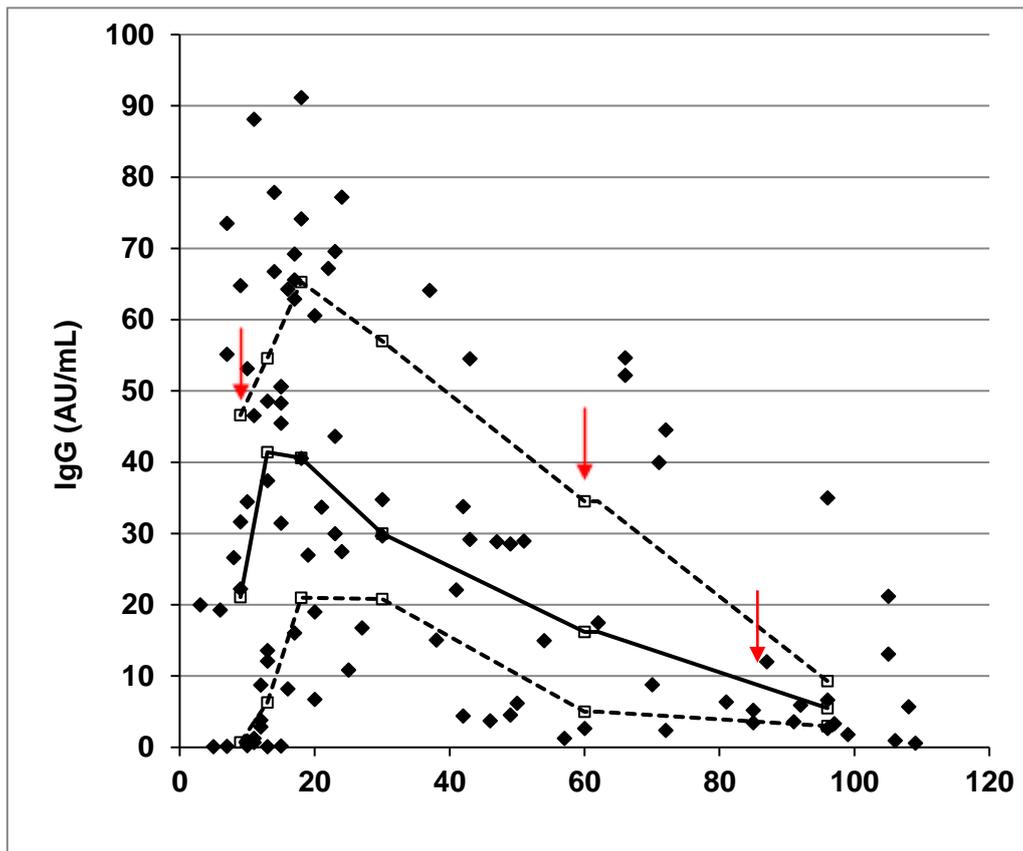
**Figure 1.** Correlations between Maglumi and Architect SARS-CoV-2 IgG levels

The trend line represent the Passing-Bablok correlation [Architect= 0.067 (0.045/0.096) Maglumi + 4.5 (3.3/5.2)]

The case study was subdivided into 6 groups ( $\leq 11$  days from the onset of symptoms, 12-15 days, 16-21 days, 22-43 days, 46-72 days and 81-109 days), homogeneous by number (from 15 to 18 specimens per group).

The clinical sensitivity in the first 3 groups was 66.7%, 87.5%, 100.0% for Maglumi and 66.7%, 81.2%, 93.3% for Architect. In the fourth and fifth group the sensitivities were 100% for both methods and in the last group was 100% for Architect and fell to 87.5% for Maglumi.

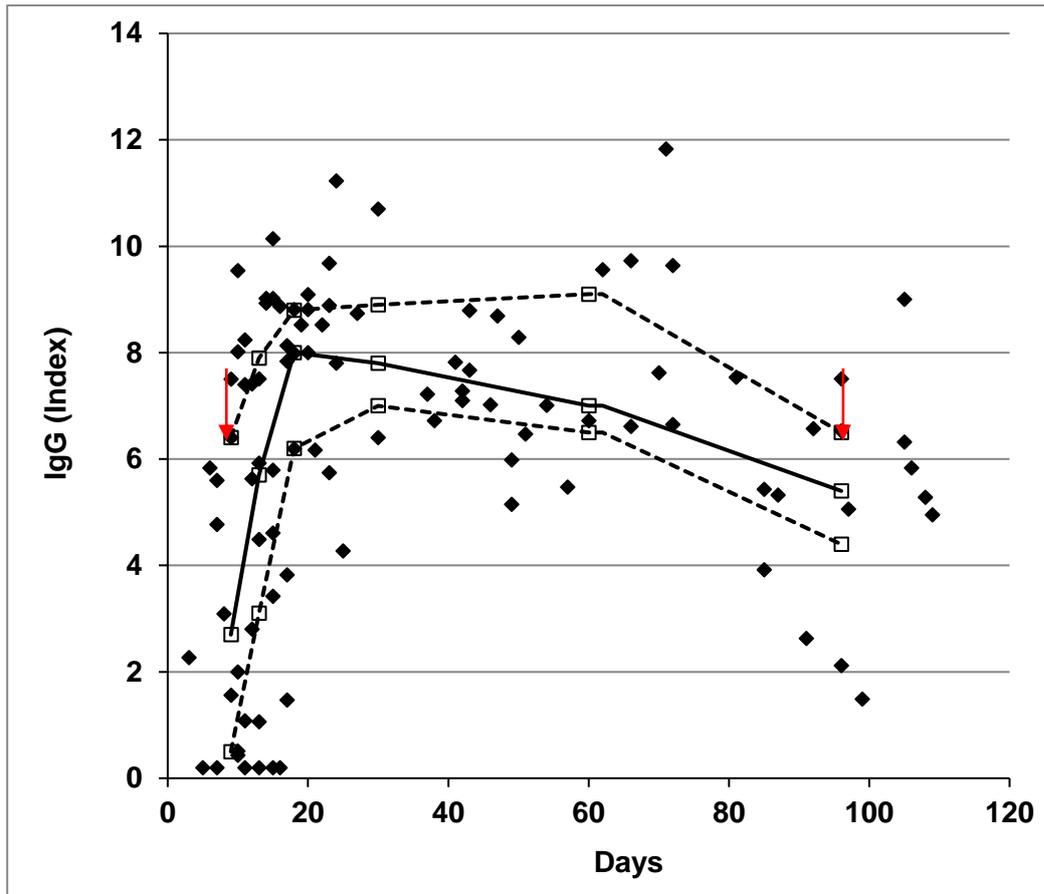
Considering the quantitative results in the samples, after a rapid increase up to about 20 days, we can see a subsequent decrease of the mean concentrations for the levels measured by Maglumi (figure 2).



**Figure 2.** Distribution of IgG levels of the single specimens measured by Maglumi in relation to the days since the onset of symptoms.

*In abscissa are reported the days from the onset of symptoms, in ordinate the concentrations of IgG Maglumi. The solid lines connect the median concentrations of IgG of each class, the dotted lines the respectively 25°-75° percentile. Red arrows represent the classes of cases significantly different from that with higher concentrations (Kruskal-Wallis test  $p=0.0005$ ).*

The same specimens measured by Architect showed a similar increase but a more stable behavior, with a modest decrease only after about 90 days (fig.3).



**Figure 3.** Distribution of IgG levels of the single specimens measured by Architect in relation to the days since the onset of symptoms.

*In abscissa are reported the days from the onset of symptoms, in ordinate the concentrations of IgG Architect. The solid lines connect the median concentrations of IgG of each class of cases, the dotted lines the respectively 25°-75° percentile. Red arrows represent the classes of cases significantly different from that with higher concentrations (Kruskal-Wallis test  $p=0.0002$ ).*

#### 4. Discussion

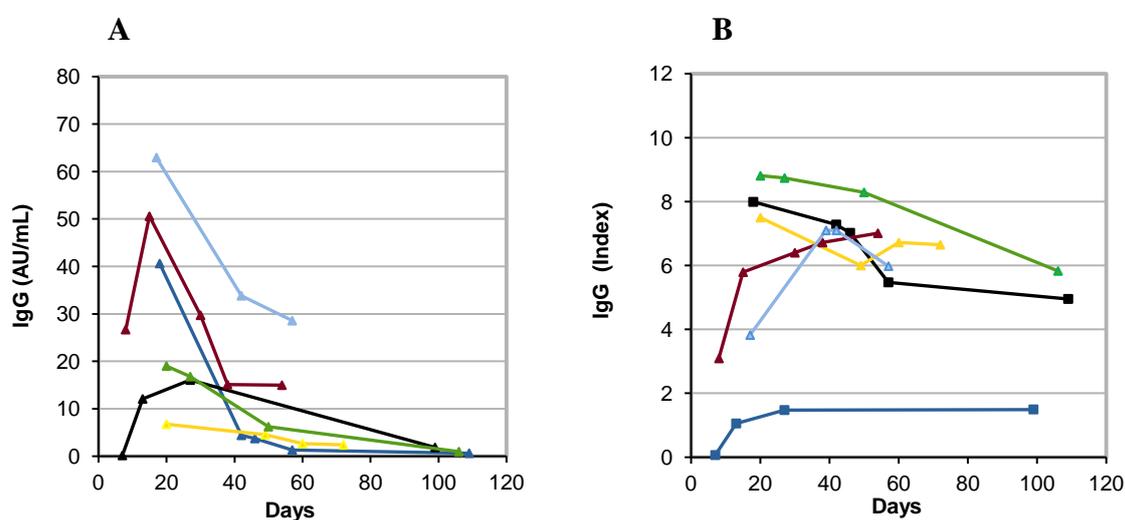
The persistence of antibodies against SARS-CoV-2 is not known. Studies on the immune response to other coronaviruses could aid in predicting a possible trend. Concentrations of IgG were found to decline after few months from the onset of symptoms, although the positivity rate remained relatively stable over a longer period [14,15]. A model of antibodies kinetics [16] mainly based on previous experience from other coronaviruses predicted a peak around 2-4 weeks and a subsequent slow decrease of antibody titer, with the hypothesis that about 50% of cases will be negative one year after the infection.

In the present study, we evaluated the correlation between two different automated high throughput methods for the IgG determination. In particular, the Maglumi test was previously evaluated only in comparison with an ELISA test [5,6]. Moreover, the time course of the antibodies response to SARS-CoV-2 was studied in some patients for a longer time than previously reported, to the best of our knowledge. Our data seems to confirm both the hypothesis of a precocious decrease of the titer of the antibodies against SARS-CoV-2, and the findings of the only study that to date followed-up a number of patients for about 2-3 months, showing a decrease of IgG concentrations [17]. However, the behaviour of the antibodies kinetics seems different when measured with different methods. The correlation between Maglumi and Architect methods, although statistically significant, showed a very disperse distribution of cases. This results could be

expected, since the two methods measured antibodies against different virus proteins, although the Maglumi detect antibodies directed against both spike and nucleocapside proteins. Moreover, different expression of the results were used in the different methods. In fact, statistically satisfactory but conflicting quantitative correlations between methods were already previously reported [2].

Anyway, when measured by Maglumi, the antibody concentrations showed a rapid decline, significant already after 45 days, and in the class with specimens from 81 to 109 days after the onset of symptoms the sensitivity drop to 87% from 100% and the median concentrations of IgG were less than 15% compared to the levels found after about 20 days. On the other end, the same samples measured by Architect showed a quite constant trend up to 80 day, and then a moderate decline, with the positivity rates that did not fall below the 100%.

A limitation of this study is that more than a half of the patients were represented by only one sample. To extrapolate the trend, we included specimens at different times obtained from different patients. However, in the 6 patients with at least 3 samples collected at least up to 50 days after the onset of symptoms, the parallel determination with the two methods confirms an evident decrease over time for Maglumi, and a constant trend or a hinted decline for Architect (figure 4).



**Figure 4.** Spaghetti plot of the 6 samples with at least 3 withdrawal in more than 50 days from the onset of symptoms, measured by Maglumi (A) and Architect (B).

Another limitation could be the lack of the comparison with the neutralization test. However, both these type of antibodies seems to correlates with neutralizing antibodies responses [2,18].

In this study, we determined antibody concentrations with two methods that measure a different mix of antibodies against the main immunogenic proteins of the virus. The quantitative differences between method should be taken into careful considerations, in particular for the possible discordant results in the mean-long term respect to the onset of the symptoms. Moreover, our data could suggest an unstable immune response to SARS-CoV-2 infection, more or less evident depending on the type of the antibodies measured and the methods used for the determination. All this should be considered by the laboratories, and could have consequences in carrying out epidemiological studies, in the evaluation of the time of collection of hyperimmune plasma for convalescent plasma therapy, as well as in the prediction of postinfection immunity.

**Author Contributions:** Conceptualization, R.D. and P.C.; sample collection and methodology, R.D. and H.A.; formal analysis, R.D.; writing—original draft preparation, R.D.; writing—review and editing, R.D., H.A. and P.C.; supervision, P.C. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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