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Short communication

Seroconversion panels demonstrate anti-SARS-CoV-2 antibody development after administration of the mRNA-1273 vaccine

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ABSTRACT

Seroconversion panels are an important tool for investigating antibody responses in acute and chronic phases of disease and development of serological assays for viral diseases including COVID-19. Globally it is anticipated that vaccines against SARS-CoV-2 will facilitate control of the current pandemic. The two COVID-19 seroconversion panels analyzed in this study were obtained from healthcare workers with samples collected before vaccination with the mRNA-1273 vaccine (Moderna) and after the first and second doses of the vaccine. Panel samples were tested for antibodies to SARS-CoV-2 (IgG). Individual subjects with a positive response for anti-SARS-CoV2 IgG in their pre-vaccination samples showed a significantly enhanced response to the first vaccination. In older subjects, lower immunological responses to the first injection were observed, which were overcome by the second injection. All subjects in the study were positive for anti-SARS-CoV-2 IgG after the second dose of vaccine.

1. Introduction

Since December 2019, COVID-19, the disease resulting from SARS-CoV-2 infection, has affected >404 million people worldwide and caused >5.7 million deaths [1]. Vaccines developed against SARS-CoV-2 [2,3] have provided the healthcare community with crucial tools that can ultimately bring the pandemic under control. In this paper, the seroconversion panels with samples collected before and after administration of a SARS-CoV-2 vaccine are described that demonstrate the immune response to vaccination.

Seroconversion panels are serial blood samples collected before and after the antibody development in response to viral infection or vaccination. They can be useful tools in the creation of antibody assays, determination of the window period of detection, validation and quality control for development and manufacture of commercial antibody tests. Detection of viral exposure and vaccine effectiveness is a critical step in gaining control of SARS-CoV-2. The panels are also a source of well-defined neutralizing antibodies useful in investigating their effectiveness blocking new COVID-19 variants.

The seroconversion panels analyzed in this study were collected from two groups of subjects before vaccination (mRNA-1273) and after the first and second doses of the vaccine. The samples were analyzed using a chemiluminescent immunoassay (CLIA) and an enzyme-linked immunosorbent assay (ELISA).

2. Materials and methods

The samples in these seroconversion panels were collected from consenting healthcare workers at a hospital (Tennessee, USA). This study was conducted under an approved IRB protocol ([1149706-4] Diagnostic QC and Pre-Clinical Sample Collection Project) and in compliance with all applicable regulatory guidelines.

Two seroconversion panels were used to characterize the appearance of anti-SARS-CoV-2 IgG after administration of the mRNA-1273 SARS-CoV-2 vaccine: 15 subjects in COVID-19 Vaccine Panel G and 30 subjects in COVID-19 Vaccine Panel H (Access Biologicals, Vista, CA, USA). Samples were collected prior to the first vaccination (objective target ≤2 days), prior to the second vaccination (objective target ≤2 days), and after the second vaccination (objective target 13–15 days). The samples were either serum sam-

Abbreviations: CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; EDTA, Ethylenediaminetetraacetic acid.

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ples collected in serum separating tubes (Panels G and H) and/or plasma samples collected in the presence of potassium EDTA (Panel H). Samples were stored at −20 °C until use. Samples were thawed at room temperature and gently mixed by inversion prior to testing.

Panel G was undiluted, unpreserved serum specimens collected from 15 subjects between 22nd December 2020 and 25th February 2021. The subjects were healthy adults 21–76 years old who received two injections of mRNA-1273 vaccine (100 μg - objective target 28 days apart). There were 5 male and 10 female subjects in this group. All subjects were Caucasian.

Samples in Panel G were serum divided into 1 mL aliquots. The samples in Panel G were tested by CLIA (Liaison SARS-CoV-2 IgG Assay, Diasorin, Inc, Saluggia, Italy: EUA approved) and ELISA (Progenika anti-SARS-CoV-2 IgG kit, Progenika Biopharma, Derio, Bizkaia, Spain: CE-IVD certified immunoassay). Manufacturer data showed the CLIA had 98.5% specificity (95% CI 97.5%-99.2%) in samples from 1000 blood donors. Concordance of the CLIA with a plaque reduction neutralization was 97.8% (95% CI 94.4–99.1%) on negative samples and 94.4% on positive samples (95% CI 88.8–97.2%) [4]. For the ELISA, the manufacturer showed the negative percent agreement was 99% (95% CI 97–99%) in 480 serum samples from individual donors and the positive percent agreement was 97% (95% CI 90–99%) for 65 samples from patients with confirmed SARS-CoV-2 (by RT-PCR) collected 15 or more days after symptoms onset [5].

Panel H was comprised of undiluted, unpreserved serum and potassium EDTA-treated plasma specimens collected from 30 subjects between 23rd December 2020 to 15th March 2021. The subjects were healthy adults 19–73 years old who received two injections of mRNA-1273 vaccine (100 μg - objective target 28 days apart). There were 9 male and 21 female subjects. The subjects were 29 Caucasians and one African American. Samples from this panel were tested using the CLIA.

The CLIA and ELISA SARS-CoV-2 IgG assays were performed according to the manufacturers' directions. Both utilize recombinant antigens specific to SARS-CoV-2: CLIA - antigens to S1 and S2 spike protein IgGs; ELISA - antigens to S1 spike protein IgG. The specific composition of the antigens was not specified and are presumed to be nonidentical.

A kappa correlation was performed to compare the qualitative values between CLIA and ELISA assays. Quantitative variables were compared using Mann-Whitney-Wilcoxon. These analyses were conducted for comparisons between two groups (e.g., naïve versus convalescent participants) (JMP software 16.0, Cary, NC, USA).

The vaccine administered to Panel G and Panel H subjects was the mRNA-1273 SARS-CoV-2 vaccine (Moderna, Cambridge, MA, USA), a lipid nanoparticle-encapsulated mRNA vaccine. The mRNA in this vaccine encodes for the perfusion-stabilized full-length SARS-CoV-2 spike protein. This vaccine has been proven to be highly efficacious in a phase 3 randomized, observer-blinded, placebo-controlled clinical trial – preventing 94% of Covid-19 illness in treated subjects compared to the placebo group. [2].

3. Results

Testing of pre-vaccination samples in Panel G (n = 15 subjects) with the CLIA gave the following results (Table 1, Fig. 1A: First Point): 13 negative, 2 positive (Subjects 5 and 15). The ELISA gave similar results (Table 1, Fig. 1B: First Point): 12 negative, 2 positive (Subjects 5 and 15) and 1 equivocal (Subject 10). The presence of anti-SARS-CoV-2 IgG in two subjects indicates that they were infected with the virus prior to sample collection.

The samples in Panel G collected after the first injection of the mRNA-1273 vaccine showed 13 positives and 2 negatives using the CLIA (Table 1, Fig. 1A: Middle Point). The ELISA showed 14 positives and 1 negative after the first vaccine injection (Table 1, Fig. 1B: Middle Point). The highest antibody responses after the first injection in both assays were in the two subjects who had detectable pre-vaccination antibodies (Subjects 5 and 15). The negative samples were from two of the oldest subjects (Subjects 3 and 4: 73 and 76 years old).

Samples collected after the second vaccine injection were positive in all 15 subjects in both assays (Table 1, Fig. 1A: Third Point and Fig. 1B: Third Point). There was good agreement between the CLIA and ELISA assays (Kappa coefficient = 0.900–0.947; considering one equivocal ELISA value as positive or negative, respectively).

With Panel H (n = 30 subjects), pre-vaccine samples showed 24 negatives and 6 positives (Table 2, Fig. 1C: First Point). Subject 19 was near the cut-off and was counted as negative. The six positive pre-vaccination samples were in Subjects 2, 8, 12, 16, 24 and 30. These samples indicate previous COVID infections. The samples in Panel H were tested using only the CLIA.

The samples collected after the first vaccine dose showed 25 positives and 5 negatives. (Table 2, Fig. 1C: Middle Point). Six positives (Subjects 2, 8, 12, 16, 24 and 30) showed a marked increase in IgG values and corresponded to subjects with positive pre-vaccination samples. The negative samples were from two of the oldest subjects (Subjects 18 and 21: both 73 years old) and the other three from people >54 years (Subjects 5, 10 and 25: 58, 56 and 55 years old, respectively).

The Panel H samples collected after the second vaccination were positive for all 30 subjects (Table 2, Fig. 1C: Third Point).

Fig. 2 shows the immune response for all subjects (both panels). The data are grouped into naïve subjects (negative pre-vaccination samples) and COVID-19 convalescent subjects (positive pre-vaccination samples). The pre-vaccine samples showed a significant difference between the groups (mean ± SD: naïve 4.2 ± 1.8 arbitrary units (AU/mL) versus convalescent 131.2 ± 157.9 AU/mL; $p < 0.0001$). Samples collected after the first vaccination showed significantly higher IgG levels (>400 AU/mL versus 108.1 ± 82.8 AU/mL; $p < 0.0001$) in convalescent subjects compared to naïve subjects. Immunological responses after the second vaccination were similar - no significant differences between the groups (363.7 ± 81.8 AU/mL in naïve subjects and > 400 AU/mL in convalescent subjects; $p = 0.1541$).

The comparison of the immunological response after the first vaccination between younger naïve subjects (<54 years; n = 20; 137.4 ± 56.2 AU/mL) and older naïve subjects (≥54 years; n = 15; 61.7 ± 78.0 AU/mL) showed a significant difference ($p = 0.0024$) using results from both panels. Convalescent subjects were not included in this analysis since the aim was to assess any age-related difference in antibody generation and these subjects had antibodies present before vaccination. Moreover, seven of the 15 older naïve subjects did not generate an immune response after the first vaccination while all the younger naïve subjects generated an immune response.

4. Discussion

The measurement of IgG levels is a crucial tool in monitoring and controlling the spread of COVID-19 and in determining medication and vaccine effectiveness. Detection of antibodies by immunoassay is useful for the verification of humoral immunity whether due to past infection, passive immunity (e.g., convalescent plasma or monoclonal antibodies) or vaccination.

A seroconversion panel can be used for the creation, development and production of antibody immunoassays. In fact, some regulatory authorities have issued guidelines requiring or recommending the use of seroconversion panels in the validation

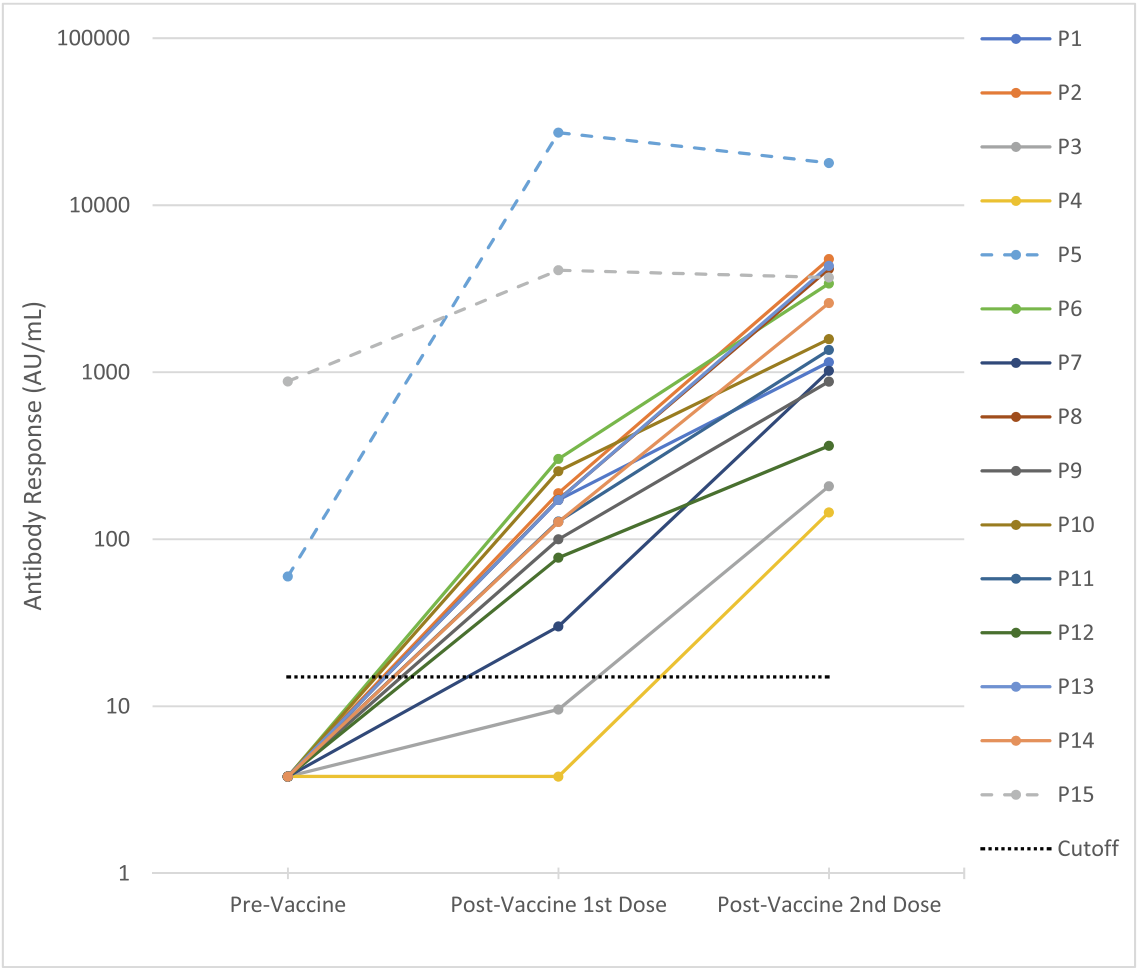
Table 1
Results of antibody presence before vaccination (Pre-vaccine) and Post-vaccine (after the 1st dose and after the 2nd dose) of the subjects in Panel G (n = 15).

			Post-Vaccine	
Detection method	Result	Pre vaccine	1st dose	2nd dose
CLIA	Positive	2	13	15
	Negative	13	2	0
ELISA	Positive	2	14	15
	Negative	12	1	0
	Equivocal	1	0	0

of serological assays for detection of viral infection [6]. Seroconversion panels are available for several viral diseases: hepatitis A, hepatitis B, hepatitis C, and human immunodeficiency virus (HIV).

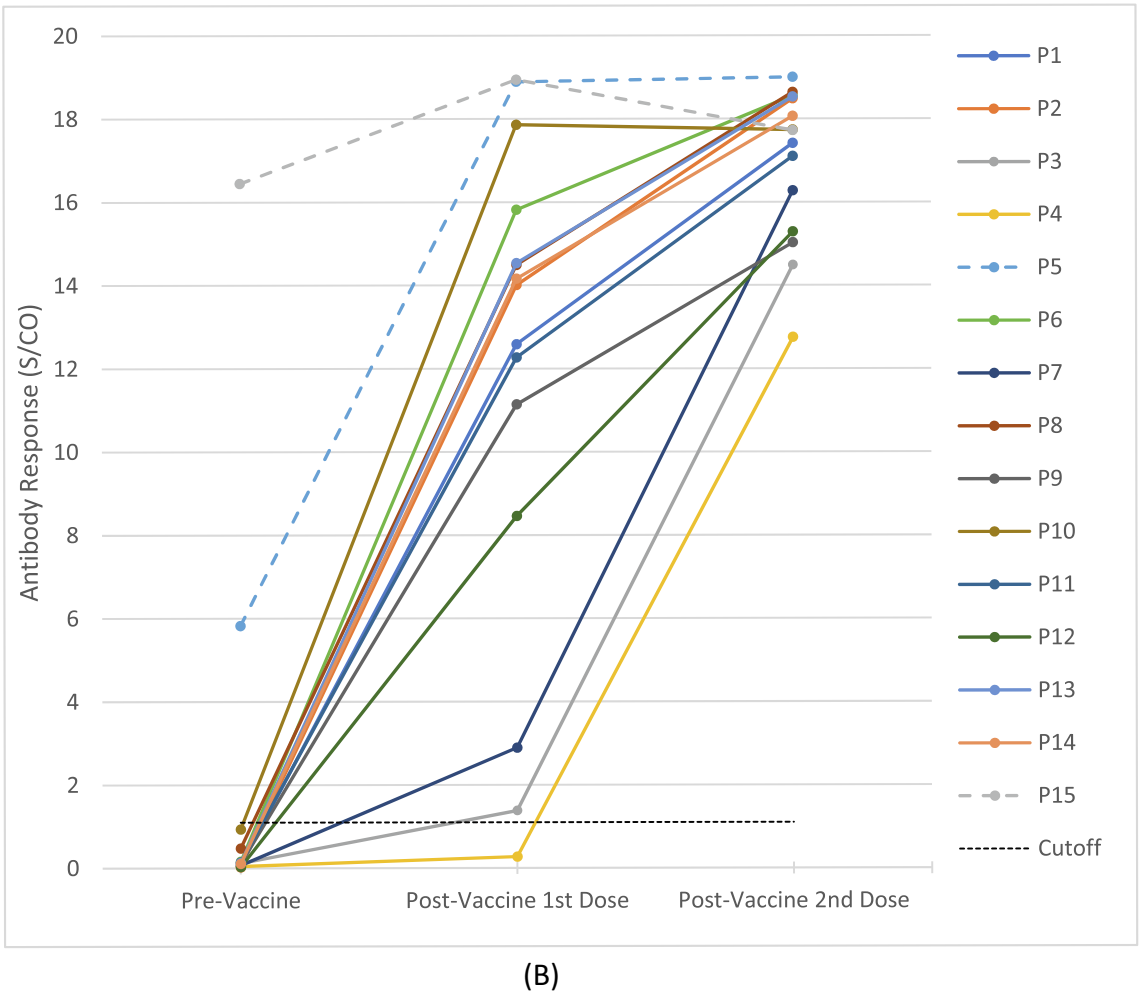
A seroconversion panel from a single subject who had community-acquired COVID-19 has been previously described [7]. The previously described seroconversion panel was the result of detection of SARS-CoV-2 infection in a regular plasma donor. This frequent donor had several pre-infection samples available from previous donations. Pre-infection samples are not available for most COVID-19 patients as blood is usually not collected until treatment is initiated.

Because the seroconversion panels described in this paper were centered around vaccination, pre-conversion samples were available for most of these subjects. Some subjects in each panel (n = 2 Panel G and n = 6 Panel H) were positive for anti-SARS-CoV-2 IgG prior to vaccination. These results indicate these subjects had COVID-19 infections prior to collection of these samples. Subjects with positive pre-vaccination samples had the highest



(A)

Fig. 1A. Antibody responses (IgG) in a seroconversion panel (Panel G) of 15 subjects before and after vaccination with two doses of the mRNA-1273 SARS-CoV-2 vaccine. These results were obtained using a chemiluminescent immunoassay (CLIA). Responses ≥15.0 units were considered positive (cutoff).



(B)

Fig. 1B. Antibody responses (IgG) in Panel G before and after vaccination. These results were obtained using an enzyme-linked immunosorbent assay (ELISA). S/CO = signal-to-cutoff values. Responses ≥1.1 S/CO were considered positive.

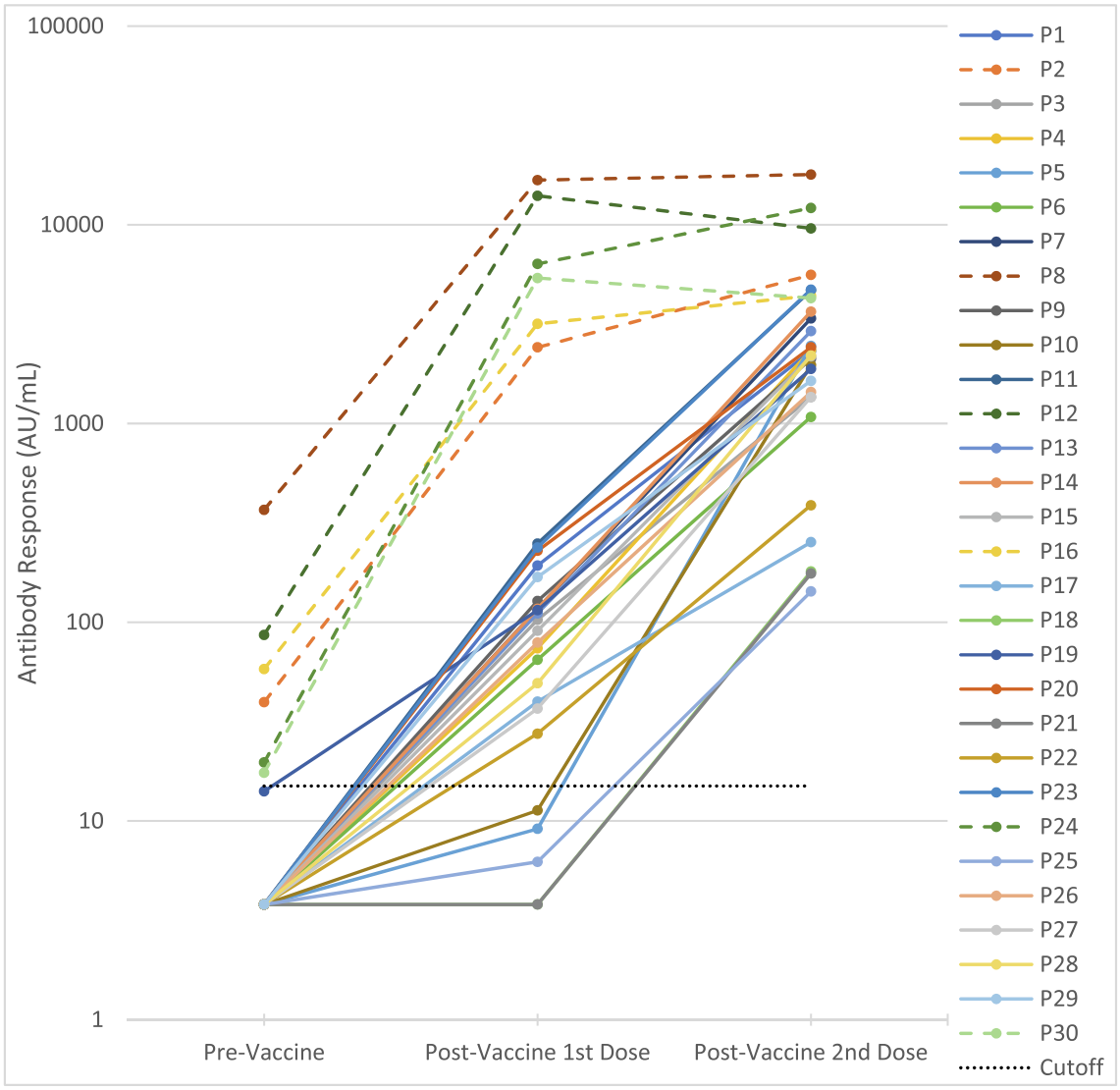
levels of IgG after the first vaccination. These results are consistent with the observation of higher antibody responses to a first vaccine dose in patients with a history of COVID-19 [8–10]. This increased response to the first vaccine has been linked to an increased level of SARS-CoV-2 antigen specific memory B cells [8].

The antibody response to the first vaccination in subjects with positive pre-vaccination samples (convalescent subjects) was of the same magnitude as that obtained with the second vaccine dose in subjects without previous COVID-19 infection (naïve subjects). This finding supports prioritizing vaccine administration based on serostatus during the current global vaccine shortage, thus maximizing coverage and effectiveness of current supplies [9–11].

Panel G was analyzed with both the CLIA and ELISA with similar results. This suggests that these seroconversion panels could be useful for comparing different immunoassays for accuracy and congruence to determine which is the most useful for a particular application.

Limitations of this study include the number of subjects studied and a racially homogenous (almost entirely white) and predominantly female population. This could limit generalization of the results to wider populations and under-represented groups. Another limitation is that the detection assays used are not neutralizing antibodies assays that would detect virus blocking antibodies. Furthermore, it would be interesting to determine the presence of specific antibodies against different antigenic parts of the virus than S1 and S2 spike proteins (e.g. nucleocapsid protein), in the group of post-vaccination samples from COVID-19 convalescent subjects.

In conclusion, these seroconversion panels demonstrate the appearance of anti-SARS-CoV-2 IgG after vaccination. They also show that convalescent subjects show enhanced responses to the first vaccination. Lower responses to the first injection – observed in four older subjects (over 72 years old) and three younger subjects (over 54 years old) – were overcome by the second injection. Furthermore, older naïve subjects (≥54 years) showed lower immune responses after the first vaccination in comparison with younger naïve subjects (<54 years). All subjects generated anti-SARS-CoV-2 IgG in response to the mRNA-1273 SARS-CoV-2 vaccine. These data show that these conversion panels could be useful tools for the development and comparison of serological tests for COVID-19 and quality control during their manufacture.



(C)

Fig. 1c. Antibody responses (IgG) in a seroconversion panel (Panel H) of 30 subjects before and after vaccination. These results were obtained using a CLIA. (AU/mL) Arbitrary Unit. Responses ≥15.0 (AU/mL) were considered positive (cutoff). The first point shows the results from samples collected prior to the first vaccination. The middle point shows the results from samples collected after the first vaccination and prior to the second vaccination. The third point shows the results from samples collected after the second vaccination. The dashed lines correspond to the convalescent participants, while the solid lines correspond to the naïve participants.

Table 2
Results of antibody presence before vaccination (Pre-vaccine) and Post-vaccine (after the 1st dose and after the 2nd dose) of the subjects in Panel H (n = 30).

			Post-Vaccine	
Detection method	Result	Pre vaccine	1st dose	2nd dose
CLIA	Positive	6	25	30
	Negative	24	5	0

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing

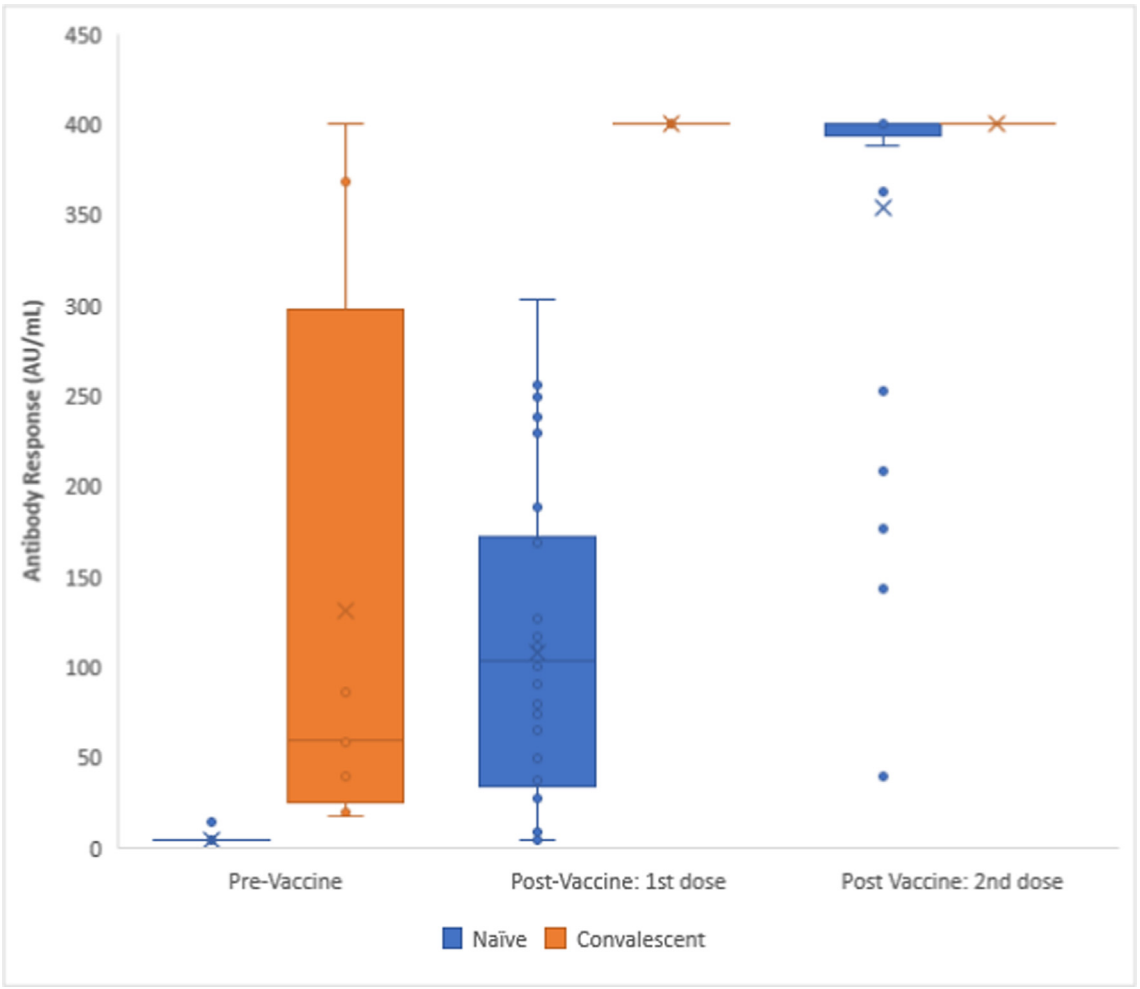

Fig. 2. Comparison of antibody responses between naïve subject versus COVID-19 convalescent subjects prior to and after vaccination. Antibody responses were measured in samples collected prior to the first vaccination, after the first vaccination and prior to the second vaccination and after the second vaccination with the mRNA-1273 SARS-CoV-2 vaccine (Moderna, Cambridge, MA, USA). These results were obtained using a chemiluminescent immunoassay (Diasorin, Inc., Saluggia, Italy). (AU/mL) Arbitrary Unit. Responses ≥15.0 (AU/mL) were considered positive; values greater than the assay range (up to 400 AU/mL) were recorded as 400 AU/mL due to the upper limit of detection. Significant differences were seen between naïve and convalescent subjects in pre-vaccine and post-vaccine: first dose samples ($p < 0.0001$).

interests: Francisco Belda, Oscar Mora, Monica Lopez Martinez and Nerea Torres are employees of Grifols. Rebecca Christie and Michael Crowlet are employees of Access Biologicals..

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