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Article

Evaluation of Serological Tests to Determine Postvaccinal Immunity to SARS-Cov-2 by Mrna Vaccines

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Abstract:

Background: The duration of the vaccine's protective efficacy against SARS-CoV-2 is unknown. Evaluation of the clinical performance of available tests is required.

Objectives: To evaluate the clinical performance of three immunoassays for the detection of IgG antibodies generated by mRNA vaccines against SARS-CoV-2.

Methods: Two automated immunoassays (Euroimmun Anti-SARS-CoV-2 ELISA IgG and Abbott SARS-CoV-2 CLIA IgG) and one lateral flow immunoassay (LFIA Test Livzon IgG) were tested. 300 samples distributed in 3 groups were analyzed: 100 subjects over 18 years old and under 45 years old, 100 subjects between 45-65 years old and another 100 over 65 years old. collected before vaccination, at 21 days, 1, 2, 3 and 6 months post-vaccination. Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and agreement (I. Kappa) were calculated for each serological test.

Results: Maximum sensitivity for IgG was 98.7%, 98.1%, and 97.8% for the ELISA Euroimmun, CLIA Abbott, and Livzon LFIA assays and maximum specificity for IgG was 99.4%, 99.9%. % and 98.4% ELISA, CLIA and LFIA respectively at 3 months after vaccination with a decrease in antibody levels from the sixth month. The best agreement was observed between ELISA and CLIA 100%; (k = 1.00). The agreement between ELIA, CLIA and LIFIA was 99% (k = 0964) at the second and third month after vaccination. Seroconversion was faster and longer lasting in the younger age groups.

Conclusion: Our study showed an equivalent and homogeneous clinical performance for IgG of three immunoassays after vaccination and that the LIFIA assay is the most cost-effective, reliable and accurate for routine use in population studies of seroconversion and seroprevalence.

Keywords: automated immunoassays; COVID-19; lateral flow immunoassay; performance; SARS-CoV-2

1. Introduction

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COVID-19 is a disease caused by the SARS-CoV-2 coronavirus whose clinical manifestations include respiratory conditions that range from the common cold to severe pneumonia with respiratory distress syndrome, septic shock, multiple organ failure, and death[1, 2].

Early detection of all cases compatible with COVID-19 is one of the key points to control transmission. The performance of Diagnostic Tests for Active Infection by SARS-CoV-2 (PDIA) should be aimed mainly at the early detection of cases with transmission capacity, prioritizing this use over other strategies. Currently, there are two active infection detection tests available, a rapid resistance detection test (Antigen Rapid diagnostic test, Ag-RDT)[3] and viral RNA detection by RT-PCR or an equivalent molecular technique[4, 5]. Detection of viral RNA by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) in respiratory tract samples is considered the standard method for detection and diagnosis in the early phase of infection[6, 7].

The vast majority of people infected with SARS-CoV-2 produce neutralizing antibodies in addition to stimulating the induction of response from memory B cells, and effector and memory CD8+ T cells [8-11]. The duration of protective immunity against the virus after natural infection is not known, but there are studies that show that vaccination of these people strengthens this protective immunity[12, 13].

Effective and safe COVID-19 vaccines have been developed that have resulted in decreased incidence, severity, and lethality of SARS-CoV-2 infection and may decrease secondary transmission[14]. Vaccines elicit high levels of anti-Receptor Binding Domain (RBD)[15] antibodies and/or directed against spike protein S[16], although it is unknown how long they protect vaccinated subjects[17], so there is concern about the efficacy and durability of the vaccine protection. Most of the tests available in clinical laboratories will detect both the antibodies generated by natural infection and those generated by the vaccine.

Different assays have been marketed: automated tests (enzyme-linked immunosorbent assays [ELISAs] or chemiluminescence enzyme immunoassays [CLIA]) or rapid screening tests (lateral flow immunoassays, [LFIA]) that can be a very useful tool to identify people who have neutralizing antibodies against SARS-CoV-2 and are potentially immune, conduct epidemiological studies, assess the effectiveness of vaccines, seroconversion and the duration of humoral immunity produced by them These tests can be easily used as point-of-care tests care or in the laboratory, with a result of less than 15 minutes. In order to be able to interpret the results of serological tests properly, the type of test being used and the neutralizing antibodies generated by the vaccine and natural infection should be taken into account. Currently approved vaccines generate S1 antiprotein antibodies. The natural infection generates anti-spicule (S) and anti-nucleocapsid (N) antibodies, so the relevance of serological tests is highly related to their clinical performance, therefore antibody tests with good sensitivity and specificity are needed, in addition to a positive predictive value, positive agreement percentage and high Cohen Kappa ratio[19-22]. In this study we intend to answer the following research question: Are immunoassays to support the diagnosis of Covid-19 disease useful for the detection of

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humoral immunity (IgG) provided by the mRNA vaccines, Comirnaty from Pfizer/BioN-Tech and of mRNA, from Moderna?.

The aim of this study was to evaluate the clinical performance of three CE-labelled immunoassays available for Europe for the detection of Ig G antibody generated by mRNA vaccines against SARS-CoV-2.

2. Materials and Methods

This prospective study included 300 healthy subjects with negative RT-PCR who were vaccinated with two injections of mRNA vaccines (Comirnaty de Pfizer/BioNTech and Moderna's mRNA vaccine) and who were assessed for postvaccine G-GIs for 6 months.

Samples were collected directly from vaccinated patients before vaccination at 21 days, 1, 2, 3 and 6 months postvaccination.

The study was carried out in the province of León in collaboration with the University of León during 2021. The subjects were selected by systematic sampling with random start of the SACYL database of 115,075 subjects vaccinated with mRNA vaccines (9.6% with modern) in the province of León to complete the 300 subjects, distributed in 3 groups: 100 subjects over 18 years old and under 45 years old 100 subjects between 45 - 65 years old and another 100 over 65 years old. Vaccinated subjects who gave informed consent to participate in the study with full vaccination dose were included, excluding those who did not sign the informed consent, with incomplete vaccination pattern, active infection and those who did not want to participate in the study.

2.2. Serological assays

Two automated immunoassays were tested (Euroimmun Anti-SARS-CoV-2 ELISA IgG against the S1 domain of the herringbone protein and the LIAISON SARS-CoV-2 S1/S2 of the herringbone protein and DiaSorin CLIA IgG and) as a gold or standard reference test and a lateral flow immunoassay (LIVZON the Diagnostic Kit for IgG Antibody to Coronavirus SARS-CoV-2 type LFIA) as a test against protein S. All three test patients included in the sample were tested.

2.2.1. ELISA test:

The Euroimmun Anti-SARS-CoV-2 ELISA IgG (Euroimmun, Lüebeck, Germany) tests were conducted according to the manufacturer's guidelines on the DS2 system, an automated microplate technology (Dynex Technologies GmbH, Denkendorf, Germany). Microplate wells are coated with recombinant S1 structural protein and the assay specifically detects IgG antibodies against SARS-CoV-2 using the S1 domain of the spike protein, including the immunologically relevant receptor-binding domain (RBD). Very useful technique for quantitative detection of anti-S1 IgG of humoral immune response not scope of seroprevalence studies or postvaccine seroconversion. It has excellent performance and good correlation with different test systems for the detection of neutralizing antibodies confirmed in other immunoassays.

2.2.2. CLIA trial

The IgG LIAISON test developed by the Italian research center DiaSorin SARS-CoV-2 S1/S2, conceived and developed in Gerenzano (Italy), guarantees extremely accurate re-

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sults, with a sensitivity of 97.4% and a specificity of 98.5%. The test is based on chemiluminescence technology (CLIA) for quantitative and qualitative analysis, IgG antibody determination against SARS-CoV-2 S1 and S2 proteins in serum or human plasma samples, without cross-reactions with other circulating human coronaviruses. This test identifies neutralizing antibodies and therefore represents an important tool for studying the immune response against SARS-CoV-2 vaccines. The units defined by the manufacturer for quantitative LIASSON test were considered negative less than 15 AU/ml and positive greater than or equal to 15 AU/ml.

2.2.3. Lateral flow test (LFIA)

IgG Antibody to Coronavirus SARS-CoV-2 test from LIVZON, a qualitative immunocolloidal lateral flow immunoassay (LFIA) that detects IgG antibodies directed at the RBD domain and against the S protein of the ear to identify people which have neutralizing antibodies against SARS-CoV-2 in serum or plasma. 10 μL were added. It is an immunocolloidal test for the qualitative detection of IgG antibodies. The results were read and interpreted 15 minutes after the test as positive or negative.

2.3. Statistical analysis

All statistical analyses were performed using the IBM software SPSS 21.0 Statistics (Statistical Package for Social Sciences, IBM Corp., Chicago, IL) and Microsoft Excel 2016. To evaluate sensitivity, specificity, positive and negative predictive values, positive and negative probability coefficient, we chose the ELISA and CLIA assays as a gold standard. Positive agreement percentage and Cohen's Kappa in all samples collected before vaccination, at 21 days, 1, 2, 3 and 6 months postvaccination. With a range between 0 and 1, a kappa value ≤ 0.40 denotes a poor agreement, a value between 0.40 and 0.75 denotes a fair/good agreement, and a value ≥ 0.75 denotes an excellent agreement. A value of $p < 0.05$ was considered statistically significant and a 95% confidence interval (CI) was reported for each metric. Sensitivity, specificity, positive predictive value (PVP) and negative predictive value (PVN), positive probability ratio (CPP) and negative probability ratio (CPN) were calculated for each serological test.

To verify the accuracy and applicability of the test in clinical practice, it must have a degree of sensitivity, specificity, general agreement and degree of concordance greater than 90%. Finally, post-vaccination seroconversion percentages were compared with all three immunological assays by age group.

3. Results

The subjects in the sample (N= 300) had a mean age of 58 ±12 years, of which 62.2% were male and 37.8% were female, with no significant differences by age and sex. By age group, 100 subjects over 18 and under 45 years (mean age 28 ±9, 100 subjects between 45 - 65 years (mean age 56 ±8) and 100 others over 65 years (mean age 83 ±9). All were healthy at the time of vaccination with negative PCR-RT and negative Ig G antibodies determined by ELISA.

Sensitivities, specificities, positive and negative predictive values, and likelihood ratios are summarized in Table 1.

Table 1. Sensitivity, specificity, predictive value and likelihood ratio of serologic assays Ig G

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ELISA assay	21 days	1 month	2 month	3 month	6 month
S % (IC95%)	60,3 (42,7-87,6)	88,6 (70,5-87,1)	95,8 (89,7-98,4)	98,7 (91,5-99,7)	83,1 (75,3-89,7)
E % (IC95%)	75,6 (66,9-82,7)	87,5 (83,891,9)	97,9 (92,1-96,6)	99,4 (96,1-99,9)	88,5 (85,3-99,6)
PPV% (IC95%)	74,8 (65,3-83,5)	97,8 (92,8-99,7)	98,3 (92,1-99,6)	99,1 (90,9-99,9)	58,3 (42,7-66,8)
PNV % (IC95%)	51,3 (43,8-58,8)	87,2 (69,3-56,4)	98,3 (92,3-99,6)	99,1 (96,6-99,9)	58,7 (42,8-78,7)
PLR (IC95%)	2,06 (1,55-4,79)	7,1 (3,1-16-9)	28,3 (15,4-44,3)	60,1(21,2-205,5)	12,1 (6,2-25,6)
NLR (IC95%)	0,6 (0,5-0,8)	0,3 (0,1-09)	0,2 (0,1-0,8)	0,1 (0,09-0,29)	0,05 (0,01-0,29)
CLIA assay	**21 days**	**1month**	**2 month**	**3 month**	**6 month**
S % (IC95%)	64,2 (46,4-92,5)	86,1 (74,5-91,1)	96,2 (87,6-97,5)	98,1 (99,9-99,5)	80,3 (73,2-88,6)
E % (IC95%)	79,1 (66,6-85,8)	89,5 (85,9-93,9)	98,5 (93,2-97,7)	99,9 (97,3-100)	86,2 (85,3-99,6)
VPP% (IC95%)	74,8 (65,3-83,5)	97,8 (92,8-99,7)	98,3 (92,1-99,6)	99,1 (90,9-99,9)	58,3 (40,5-64,7)
VPN % (IC95%)	55,6 (45,8-60,8)	88,1 (70,4-57,5)	97,9 (91,2-99,8)	99,3 (96,3-99,9)	59,8 (41,8-77,7)
PLR (IC95%)	3,3 (1,84-5,90)	8,3 (4,4-17,8)	30,2 (17,3-46,3)	72,3 (41,1-255,5)	18,1 (8,5-27,8)
NLR (IC95%)	0,5 (0,3-0,9)	0,2 (0,1-0,6)	0,15 (0,1-0,9)	0,12 (0,8-0,29)	0,03 (0,01-0,27)
LFIA assay	**21 days**	**1 month**	**2 month**	**3 month**	**6 month**
S % (IC95%)	60,6 (53,4-67-3)	80,2 (70,5-87,1)	94,8 (67,5-98,5)	97,8 (61,5-99,7)	77,6 (65,2-85,1)
E % (IC95%)	70,1 (84,8-96,3)	86,7 (63,2-79,7)	97,9 (92,1-96,6)	98,4 (85,2-99,4)	83,1 (68,6-94,3)
VPP% (IC95%)	75,9 (68,3-82,2)	96,1 (94,8-98,7)	98,1 (92,1-99,6)	97,1 (90,9-99,2)	48,5 (12,3-191)
VPN % (IC95%)	53,3 (44,4-60,9)	87,9 (91,3-99,6)	98 (92,3-99,6)	96 (89,6-98,7)	48 (12,8-189)
PLR (IC95%)	2.1 (1,52-2,70)	9,1 (8,56-10,62)	30,1 (12,8-96,2)	72,6 (43,1-96,2)	13,1 (6,4-26,9)
NLR (IC95%)	0,56 (0,46-0,69)	0,3 (0,1-0,5)	0,2 (0,08-0,4)	0,1 (0,7-0,3)	0,05(0.0-0.3)

S: Sensitivity; E: Specificity; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio; ELISA (Euroimmun Anti-SARS-CoV-2 ELISA IgG9; CLIA (LIAISON® SARS-CoV-2 S1/S2 IgG test); LFIA (LIVZON Test IgG Antibody to Coronavirus SARS-CoV-2)

3.1. Sensitivity

The IgG ELISA sensitivity was 60.3%, after the first dose, increased to 95.8% 2 months after vaccination, was maximum at 3 months with 98.7% and decreased to 83.1% from 6 months. The sensitivity for CLIA was very similar, increasing from 60.4% to 86.1%, 96.2% and 98.1% until the third month, decreasing to 80.3% from the sixth month. The sensitivity of LFIA behaved similarly, increasing from 60.6% to 80.2%, 94.8% and 97.8% until the third month, decreasing to 83.1% from the sixth month. The general sensitivity for post-vaccine IgG was equivalent (95%) for ELISA, CLIA and LFIA, being maximum between the second and third post-vaccination months, declining from the sixth month coinciding with the decrease in the level of protective antibodies. Comparison of susceptibility during the first 21 days of vaccination did not reveal any significant differences between the three assays being significant from one month ($P < 0.05$).

3.2. Specificity

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The general specificity was equivalent for ELISA, CLIA and LFIA (higher than 98), being maximum between the second and third post-vaccination months, declining from the sixth month coinciding with the decrease in the level of protective antibodies. The specificity was significantly different between the three trials from the beginning ($p<0.05$). In addition, the three trials have very homogeneous values overall of more than 90% in the positive and negative predictive values, being these maximum between the month and the 3 months.

3.3. Likelihood ratio

The positive likelihood ratio increases steadily between day 21 and three months, being higher between month 2 and month 3 for CLIA and LIFIA than for ELISA.

3.4. Concordance of Ig G serologic tests.

Table 2 summarized the general agreement and agreement regarding the timing of IgG determinations from the start of vaccination to 6 months. Overall an excellent agreement was observed between the three trials even from day 21 of vaccination. The greatest concordance was observed between ELISA and CLIA at 100%; (k=1.00) in the second and third months of vaccination.

Table 2. Concordance of Ig G serologic tests.

Concordance	Assays	ELISA% (Kappa)	CLIA%(Kappa)
General	**CLIA**	97% (0,938)	
	LIFIA	96% (0,919)	97% (0,941)
21 days	**CLIA**	88% (0,666)	
	LIFIA	95% (0,914)	91% (0,753)
1 month	**CLIA**	96% (0,931)	
	LIFIA	96% (0,919)	98% (0,954)
2 month	**CLIA**	100% (1.00)	
	LIFIA	99% (0,964)	99% (0,964)
3 month	**CLIA**	100% (1.00)	
	LIFIA	99% (0.964)	99% (0,964)
6 month	**CLIA**	87% (0,625)	
	LIFIA	93% (0,845)	91% (0,812)

ELISA (Euroimmun Anti-SARS-CoV-2 ELISA IgG9; CLIA (LIAISON® SARS-CoV-2 S1/S2 IgG test); LFIA (LIVZON Test IgG Antibody to Coronavirus SARS-CoV-2)

3.4. Immune response.

The immune response generated by RNAm vaccines was determined with the three immunoassays in the different age groups. As shown in Figure 1, younger patients showed a faster and more durable response than patients over 65 years of age throughout the follow-up period.

Figure 1. Proportion of seroconversions by age group

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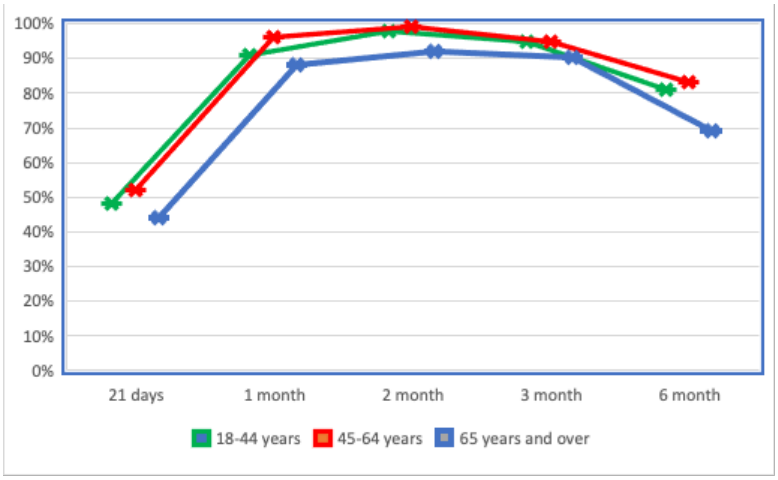

This figure shows the immune response against mRNA vaccines in the age groups, observing how it is less lasting in the group over 65 years of age.

4. Discussion

The clinical performance of tests for the detection of IgG antibodies produced by vaccines is critical to knowing the immune status of the population and the duration of immunity.

It should be noted that the lack of scientific data at this time still does not allow to determine whether IgG neutralizing antibodies against SARS-CoV-2 provide long-term immunity to the virus or whether they protect patients against reinfection. By blocking the entry of the virus into the cell, neutralizing antibodies can block viral replication: this does not eliminate the need for a PCR test to confirm that the patient is not infectious.

In this study, we evaluated three different commercial immunoassays with CE marking for the detection of SARS-CoV-2 antibodies in human serum and plasma. The ELISA test was performed in a semi-automatic microplate technology that requires high handling and limited testing capacity per day (90 tests every 4 hours).

In contrast, the CLIA trial is a fully automated random access test that can perform more than 4,000 tests every 24 hours. These two trials are used in clinical laboratories, unlike LFIA, which can be used as a point-of-care test or in clinical laboratories and provides a result in 15 minutes.

The performance of the Euroimmun trial has been evaluated in some studies showing sensitivity for IgG between 85 % and 95 % after natural infection and specificity between 95 % and 100 %[19,23-25]. Other studies reported the clinical performance of the Abbott trial[21 24, 25].

Sensitivity to IgG was between 94% and 100% after natural infection and specificity between 99% and 100%. The LFIA test had 95% sensitivity and 97% specificity for diagnosis. In our study, we showed maximum sensitivity for IgG of 98.7%, 98.1% and 97.8% for the ELISA Euroimmun, CLIA Abbott and Livzon LFIA tests at 3 months of vaccination. A maximum specificity for IgG of 99.4%, 99.9% and 98.4% for ELISA, CLIA and LFIA respectively at 3 months of vaccination with a decrease in antibody levels from the sixth month.

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Although there are many LFIA with CE marking on the market. Two studies showed that sensitivity and specificity were similar to those of the Euroimmun assay[19, 26] . However, as far as we know, only one study compared clinical performance between CLIA Abbott and LFIA[27] and no study described the diagnostic performance of LIVZON.

The best agreement was observed between ELISA and CLIA 100%; (k = 1.00) at the second and third month of vaccination. The agreement between ELIA, CLIA and LIFIA was 99% (k = 0964) at the second and third month of vaccination.

Another interesting finding was the homogeneity of the response of IgG antibodies in patients vaccinated against protein S and S1, as demonstrated by studies carried out with other quantitative and qualitative immunoassays[19,28]. These results are consistent with those obtained in another study where two automated immunological assays were performed (Abbott trials SARS-CoV-2 CLIA IgG and Euroimmun Anti-SARS-CoV-2 ELISA IgG/IgA) and a lateral flow immunoassay (LFIA NG-Test IgG-IgM COVID-19), in which sensitivity for IgG detection was 100.0% for all trials. The overall specificity of IgG was higher for CLIA and LFIA (more than 98 %) compared to ELISA (95,8 %). The best agreement was observed between the CLIA and LFIA tests (97 %; k = 0.936), so this study shows that the NG-Test lateral flow test is reliable and accurate for routine use in clinical practice for detection of post-vaccine neutralizing Ig G antibodies, as is the case with the LIVZON test in our study.

Although quantitative reference tests are the Gold standard which identifies the antibodies generated by the vaccine, determine the IgG Anti SARS-CoV-2 antibodies against the binding domain of the S1 receptors. A strong correlation between RBD-binding antibody levels and SARS-neutralizing antibodies has been observedCoV-2 in patients who support the use of RBD antigen in serological diagnostic tests and RBD-specific antibody levels as a correlate of neutralizing SARS-CoV-2 antibodies in people[30], the advantages of LFIA are the ability to reach larger population groups when used in care, and to assess collective immunity without saturating laboratory capacity and even very low cost, so it makes this immunoassay the most cost-effective. Color intensity in line regions was correlated with SARS-Cov-2 antibody concentration. However, these devices should be used with caution. Trained personnel or automated reading devices are needed for a good interpretation of the result to avoid errors in the interpretation of the results in situations that are not under the control of trained personnel. For this reason, developing automated readers could help reduce errors and increase sensitivity. In addition, such a device could support the transmission of results to a public health institution to provide real-time information on seroprevalence at the population level and improve the traceability of results.

An important limitation that was a pilot trial of 300 subjects and does not allow its generalization to the general population, but to evaluate the seroconversion of previously healthy vaccinated patients in a given province.

Another limit is the determination of neutralizing antibodies from RNAm vaccines. Neutralizing antibody titers correlate with anti-SARS-CoV-2 S antibody titers (arranged in regions S1, RBD and S2) however, to determine this correctly, tests are required in third-level laboratories that exceed the capacity of this study.

5. Conclusions

To conclude, our study showed equivalent clinical performance for IgG of three immunoassays (ELISA, CLIA and LFIA) 21 days post-vaccination. The three trials had, as expected, a low sensitivity after the first dose at 21 days increasing progressively from the second dose, being maximal during the second and third post-vaccination months to slowly decrease to the sixth month, as neutralizing antibodies to SARS-CoV-2 decrease. Therefore, serological tests may be useful to confirm past COVID-19, to make epidemiological seroprevalence studies, to know seronversion rates, the duration of immunity conferred by vaccines and help establish correct immunization guidelines. Our results are

consistent with those of other studies that suggest that post-vaccination antibody response testing is an important and feasible tool for following people after vaccination and selecting people who might require additional doses of vaccine or people who may not need more doses due to a previous SARS-CoV-2 infection. It is currently unclear whether IgG antibodies are protective against reinfection.

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Graciela Iglesias García and Ángel Díaz Rodríguez participated in the drafting, coordination of the manuscript, as well as in the critical review of the different versions until this final version.

All authors approved the final version of the manuscript for publication and guarantee that all aspects of the manuscript have been reviewed and discussed among all in order to be exposed with maximum precision and integrity.

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References

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Feb 20;382(8):727-733.

[2] Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020 May;8(5):475-481.Erratum in: Lancet Respir Med. 2020 Apr;8(4):e26.

[2] Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al; Cochrane COVID-19 Diagnostic Test Accuracy Group. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2020 Aug 26;8(8):CD013705.

[4] Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Evaluation of SARS-CoV-2 serology assays reveals a range of test performance. Nat Biotechnol. 2020 Oct;38(10):1174-1183.

[5] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK et al; Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3):2000045.

[6] Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections - the state of the art. Emerg Microbes Infect. 2020 Dec;9(1):747-756.

[7] Holgersen EM, Gandhi S, Zhou Y, Kim J, Vaz B, Bogojeski J, et al. Transcriptome-Wide Off-Target Effects of Steric-Blocking Oligonucleotides. Nucleic Acid Ther. 2021 Dec;31(6):392-403.

[8] Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021 Feb 5;371(6529):eabf4063.

[9] Shurin MR, Morris A, Wells A, Wheeler SE. 2020. Assessing immune response to SARS-CoV-2 infection. *Immunotargets Ther* 9:111–114.

[10] Ju B, Zhang Q, Ge J, Wang R, Sun J, Ge X, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature. 2020 Aug;584(7819):115-119.

[11] Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell. 2020 Jun 25;181(7):1489-1501.e15.

[12] Suthar MS, Zimmerman MG, Kauffman RC, Mantus G, Linderman SL, Hudson WH et al. Rapid Generation of Neutralizing Antibody Responses in COVID-19 Patients. Cell Rep Med. 2020 Jun 23;1(3):100040.

[13] Hall VJ, Foulkes S, Charlett A, Atti A, Monk EJM, Simmons R, et al. SIREN Study Group. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). Lancet. 2021 Apr 17;397(10283):1459-1469.

[14] Ma KS, Lee CC, Liu KJ, Wei JC, Lee YT, Wang LT. Safety and Seroconversion of Immunotherapies against SARS-CoV-2 Infection: A Systematic Review and Meta-Analysis of Clinical Trials. Pathogens. 2021 Nov 24;10(12):1537.

[15] Barnes CO, Jette CA, Abernathy ME, Dam KA, Esswein SR, Gristick HB, et al. SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. Nature. 2020 Dec;588(7839):682-687.

[16]Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020 Mar 13;367(6483):1260-1263.

[17]Rastawicki W, Płaza K. The level of protective post-vaccination antibodies in NIPH-NIH employees after administration of Pfizer vaccine against COVID-19. Przegl Epidemiol. 2021;75(1):3-13.

[18] Wu JL, Tseng WP, Lin CH, Lee TF, Chung MY, Huang CH, et al. Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2. J Infect. 2020 Sep;81(3):435-442.

[19] Montesinos I, Gruson D, Kabamba B, Dahma H, Van den Wijngaert S, Reza S, et al. Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies. J Clin Virol. 2020 Jul;128:104413.

[20] Mei San Tang, Karl G Hock, Nicole M Logsdon, Jennifer E Hayes, Ann M Gronowski, Neil W Anderson, et al. Clinical Performance of Two SARS-CoV-2 Serologic Assays, *Clinical Chemistry*. Volume 66, Issue 8, 2020 August;66(8):1055–1062.

[21] Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, et al. Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. J Clin Microbiol. 2020 Jul 23;58(8):e00941-20.

[22]Zainol Rashid Z, Othman SN, Abdul Samat MN, Ali UK, Wong KK. Diagnostic performance of COVID-19 serology assays. Malays J Pathol. 2020 Apr;42(1):13-21. PMID: 32342927.

[23] Mei San Tang, Karl G Hock, Nicole M Logsdon, Jennifer E Hayes, Ann M Gronowski, Neil W Anderson, Christopher W Farnsworth. Clinical Performance of Two SARS-CoV-2 Serologic Assays, *Clinical Chemistry*, August 2020; 66(8):1055–1062.

[24] Kohmer N, Westhaus S, Rühl C, Ciesek S, Rabenau HF. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. *J Clin Virol*. 2020;129:104480.

[25] Theel ES, Harring J, Hilgart H, Granger D. Performance characteristics of four high-throughput immunoassays for detection of IgG antibodies against SARS-CoV-2 J. Clin. Microbiol. July 2020;58(8):e01243-20

 doi:10.20944/preprints202211.0086.v1

[26]Van Elslande J, Houben E, Depypere M, Brackenier A, Desmet S, André E, et al. M. Diagnostic performance of 7 rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. Clin. Microbiol. Infect. August 2020;26(8):1082-1087

[27]Dellière S, Salmona M, Minier M, Gabassi A, Alanio A, Le Goff J, et al, on behalf of Saint-Louis CORE (COvid REsearch) group. 2020. Evaluation of the COVID-19 IgG/IgM Rapid test from Orient Gene Biotech. J Clin Microbiol. July 2020; 58(8):e01233-20.

[28]Coste AT, Jaton K, Papadimitriou-Olivgeris M, Greub G, Croxatto A. Comparison of SARS-CoV-2 serological tests with different antigen targets. J Clin Virol. 2021 Jan;134:104690.

[29] Nicol T, Lefeuvre C, Serri O, Pivert A, Joubaud F, Dubée V, et al. Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of three immunoassays: Two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech). J Clin Virol. 2020 Aug;129:104511.

[30] Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann A, et al. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. Sci Immunol. 2020 Jun 11;5(48):eabc8413.