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Antibody Detection in Healthcare Workers after Vaccination with Two Doses of the BNT162b2 or ChAdOx1 Vaccine

Kyoung Ho Roh[1], Heun Choi[2], Heekyoung Choi[2], Miseon Yoon[3], Jongha Yoo[1], Yoonseon Park[4]*

[1]Department of Laboratory Medicine, National Health Insurance Service Ilsan Hospital, Goyang, Korea

[2]Department of Infectious Diseases, National Health Insurance Service Ilsan Hospital, Goyang, Korea

[3]Department of Infection Control Center, National Health Insurance Service Ilsan Hospital, Goyang, Korea

[4]Division of Infectious Diseases, Department of Internal Medicine Gil Medical Center, Gachon University College of Medicine, Incheon, Korea

***Corresponding author:** Yoonseon Park, yoonseony@gmail.com

Abstract

Background: Due to the COVID-19 pandemic, in 2020, many pharmaceutical companies have developed vaccines. To determine the efficacy of AstraZeneca's and Pfizer's vaccines, which were the first and second vaccines to be approved in Korea, respectively, we developed a method to measure their antibody-generating efficacies using immunology analyzers and a rapid antibody test available in Korea. Methods: The antibody-stimulating efficacies of the Pfizer and AstraZeneca vaccines were evaluated using Centaur® XPT SARS-CoV-2 (Siemens Healthineers, Germany), Elecsys® Anti-SARS-CoV-2 S (Roche Diagnostics, Germany), and STANDARD F SARS-CoV-2 nAb FIA (SD Biosensor, Korea). Healthcare workers were enrolled in two groups: the Pfizer (121) and AstraZeneca (117) groups. Antibody levels were measured pre-vaccination, three weeks after vaccination, and 16 weeks after vaccination. Results: The Pfizer group comprised 41 males and 80 females, while the AstraZeneca group comprised 38 males and 79 females. Antibody results were analyzed after excluding four individuals who had recovered from COVID-19. Between weeks 3 and 16, there was no significant difference (P = 0.5, 1.0) between the results of the Roche and Siemens antibody tests in the Pfizer vaccine group. However, the SD Biosensor results of comparing the Roche and Siemens antibody tests at three weeks after the initial vaccination showed a significant difference ($P < 0.0001$). Analysis of the Roche antibody test results before, at three weeks, and at 16 weeks after the administration of the Pfizer and AstraZeneca vaccines revealed significant differences between before and at three weeks after the first injection ($P < 0.0001$). Conclusion: After two doses of the Pfizer and AstraZeneca vaccines, antibody formation was above the 90th percentile of the measurement range in all subjects.

Keywords

COVID-19
Evaluation
Immunoassay
Vaccines

1. Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 [1]. Since 2020, the global pandemic of COVID-19 has led many pharmaceutical companies to develop vaccines to protect against the disease, and many more are currently developing and testing vaccines against various coronavirus strains [2-4]. As there are very limited treatments for COVID-19 infection and no oral therapies available, the current response to COVID-19 is focused on rapid diagnosis of COVID-19 using PCR testing, isolating positive cases to prevent further transmission, and reducing the number of infections using vaccination and social distancing [5,6].

The United States Food and Drug Administration (FDA) first approved Pfizer-BioNTech's COVID-19 vaccine BNT162b2 (Pfizer-BioNTech, New York, NY, United States) for emergency use on 11 December 2020, followed by Moderna's vaccine on 18 December and Janssen's vaccine on 27 February 2021 [7]. In Korea, the Ministry of Food and Drug Safety approved the use of AstraZeneca's viral vector vaccine ChAdOx1 (AstraZeneca, Cambridge, United Kingdom) on 10 February 2021, followed by Pfizer's mRNA vaccine on 5 March 2021 [8].

After vaccine development, it is common to test for neutralizing antibodies produced after vaccination to determine the effectiveness of the vaccine, although differences in infection rates between groups are sometimes calculated [9]. Methods for qualitative or quantitative testing of antibodies to coronavirus heavy S protein have been proposed, and *in vitro* diagnostic medical devices that can be tested more quickly and conveniently using automated immunoassay devices have been developed at home and abroad, and are currently available for testing with permission from the Ministry of Food and Drug Safety [10].

In this study, the results of antibody tests formed according to the type of vaccine inoculated to prevent COVID-19 infection were measured before vaccination, 3 weeks after the first dose, and 16 weeks after the first dose using automated immunoassay devices currently available in Korea and rapid antibody tests available in the field, and the specifics of automated immunoassay were presented.

2. Materials and methods

2.1. Study methods

This study was approved by the Institutional Review Board of Ilsan National Health Insurance Hospital (IRB No. NHIMC 2021-02-015) and was conducted in adults aged 19 to 69 years. From March 2021 to September 2021, 121 hospital employees who received the Pfizer vaccine and 117 hospital employees who received the AstraZeneca vaccine were studied after obtaining informed consent.

The second dose of Pfizer's vaccine was administered three weeks after the first dose, and the second dose of AstraZeneca's vaccine was administered 11 weeks after the first dose. Three whole blood samples (7 mL each) were collected in serum separator tubes (SST) 1 day before vaccination (day 0), 3 weeks after the first dose, and 16 weeks after the first dose.

The collected SSTs were immediately centrifuged at 3,500 g for 10 minutes in a refrigerated centrifuge, and the serum was collected separately using serum separator tubes and stored in a -70°C freezer until SARS-CoV-2 antibody testing. On the day of the test, the serum was removed from the freezer 30 minutes before the test and allowed to thaw to room temperature.

2.2. SARS-CoV-2 antibody test *in vitro* diagnostic device

We used three types of products licensed by the Korea Food and Drug Administration (KFDA) for *in vitro* diagnostic use. Centaur® XPT SARS-CoV-2 (Siemens Healthineers, Erlangen, Germany; hereby Centaur COV2T), Elecsys® Anti-SARSCoV-2 S (Roche Diagnostics, Mannheim, Germany; hereby Elecsys

COV2S), and STANDARD F SARSCoV-2 nAb FIA (SD Biosensor, Suwon, Korea; SD COV2) were used following the manufacturer's storage and use conditions and product instructions.

The Centaur COV2T from Siemens is a quantitative test that can be tested using the ADVIA Centaur XPT automated immunoassay (Siemens Healthineers, Erlangen, Germany) and has a measurement range of 0.6–10 indices, which converts 1 index to 1 U/mL. A result below 1.0 index is negative (non-reactive) and above 1.0 index is positive (reactive) according to the product documentation.

Roche's Elecsys COV2S is a quantitative test that can be tested using the Roche e602 automated immunoassay (Roche Diagnostics, Mannheim, Germany) with a test time of 18 minutes. It has a measuring range of 0.4–250 U/mL and a result of less than 0.8 U/mL is considered negative (non-reactive) and more than 0.8 U/mL is considered positive (reactive).

SD COV02 of SD Biosensor was developed as a rapid antibody test product, and 15 minutes after inoculation of 100 μL specimen, PI (positive index) can be checked with positive and negative results using a separate reader. A result of PI less than 20 is considered negative, and more than 20 is considered positive.

2.3. Statistical analysis

Statistical analyses were performed using MedCalc 20.111 (MedCalc Software Ltd, Ostend, Belgium). Student's *t*-test was used for continuous data and chi-squared or McNemar's test was performed for categorical data.

3. Results

3.1. Study participant information

The 121 participants in the Pfizer vaccine group consisted of 41 males and 80 females, with an age distribution of 24 aged 20 to 29 years, 24 aged 30 to 39 years, 47 aged 40 to 49 years, and 26 aged 50 to 59 years. The 117 AstraZeneca vaccine recipients were comprised of 38 males and 79 females, with an age distribution of 37 aged 20 to 29 years, 27 aged 30 to 39 years, 27 aged 40 to 49 years, 22 aged 50 to 59 years, and 4 aged 60 to 69 years (**Table 1**).

Table 1. Demographic data of Pfizer and AstraZeneca vaccine groups

Demographic data	Pfizer BNT162b2			AstraZeneca ChAdOx1		
Sex	Male	Female	Total	Male	Female	Total
	41 (33.9)	80 (66.1)	121 (100.0)	38 (32.5)	79 (67.5)	117 (100.0)
Age (yr)						
20–29	5 (12.2)	19 (23.8)	24 (19.8)	2 (5.3)	35 (44.2)	37 (31.6)
30–39	7 (17.1)	17 (21.3)	24 (19.8)	14 (36.8)	13 (16.5)	27 (23.1)
40–49	15 (36.6)	32 (40.0)	47 (38.8)	13 (34.2)	14 (17.7)	27 (23.1)
50–59	14 (34.1)	12 (15.0)	26 (21.5)	8 (21.1)	14 (17.7)	22 (18.8)
60–69	-	-	-	1 (2.6)	3 (3.8)	4 (3.4)
COVID-19 past infection						
Yes	1 (2.4)	2 (2.5)	3 (2.5)	-	1 (1.3)	1 (0.9)
No	40 (97.6)	78 (97.5)	118 (97.5)	38 (100.0)	78 (98.7)	116 (99.1)

Values are presented as n (%). Abbreviation: COVID-19, coronavirus disease 2019.

3.2. Pre-vaccination test results

Of the 121 people who received the Pfizer vaccine, three tested positive. All three were confirmed COVID-19 cases and all had recovered. Two of them had high antibody values above 250 U/mL on the Roche, Siemens, and SD Biosensor antibody tests. The other 118 people without prior infection tested negative on both the Roche and Siemens automated immunoassays, but positive on the SD Biosensor rapid antibody test in one man and one woman. Their PI values were 22.5 and 23.6, near the cut-off of 20.

One of the 117 people who received the AstraZeneca vaccine tested positive. This participant was identified as a confirmed case of COVID-19 and had recovered. He tested positive on the Roche, Siemens, and SD Biosensor antibody tests, with the Roche antibody test showing an antibody value of 65.7 U/mL, which was above the cut-off. The other 116 people who had no prior infection tested negative on both the Roche and Siemens automated immunoassays and the SD Biosensor rapid antibody test.

3.3. Test results from three weeks after vaccination

The Roche, Siemens, and SD Biosensor antibody tests were performed on 118 previously uninfected individuals who received the Pfizer vaccine. The Roche antibody test was positive in all 118, and the Siemens total antibody test was positive in 116 and negative in two. The SDI Biosensor Rapid Antibody Test was positive in 81 and negative in 37.

The Roche antibody test showed 44 (37.3%) with an antibody value of 100 U/mL or higher, while the Siemens total antibody test showed 74 (62.4%) with an index value of 5 or higher (**Table 2**). Two individuals with negative results on the Siemens total antibody test also had relatively low antibody results on the Roche antibody test, 2.48 U/mL, and 8.66 U/mL, respectively. Based on the Roche antibody results, the sensitivity of the Siemens Total Antibody Test was 98.3%. The SDI Biosensor Rapid Antibody Test had a relatively low concordance rate of 68.6% in the group with positive Roche antibody results.

The Roche, Siemens, and SDI Biosensor antibody tests were tested in 116 infection-free individuals who received the AstraZeneca vaccine. The Roche antibody test was positive in 111 and negative in 5, and the Siemens total antibody test was positive in 92 and negative in 24. The SDI Biosensor Rapid Antibody Test was positive in 45 and negative in 71.

The Roche antibody test showed 12 people (10.3%) with high antibody values above 100 U/mL, while the Siemens total antibody test showed 34 people (29.3%) with index values above 5 (**Table 3**). Of the 24 individuals with a negative Siemens total antibody test, 5 also had a negative Roche antibody test, and all 19 had relatively low antibody results of 19.99 U/mL or less. Based on the Roche antibody results, the Siemens Total Antibody Test had a sensitivity of 82.9%. The SDI Biosensor Rapid Antibody Test had a relatively low concordance rate of 39.6% in the group with a positive Roche Antibody Test result, with a cut-off of 255 U/mL for the Roche Antibody Test result above the upper limit of detection to quantitatively compare the extent of antibody formation.

3.4. Test results from 16 weeks after vaccination

Roche, Siemens, and SDI Biosensor antibody tests were performed 16 weeks after the first dose in 117 infection-free individuals who received two doses of the Pfizer vaccine, excluding one individual who was lost to follow-up after the second dose. The Roche and Siemens total antibody tests were positive in all 117, and the SD Biosensor rapid antibody test was positive in 115 and negative in 2.

The Roche antibody test showed 116 people with an antibody value of 100 U/mL or higher, 99.1%, and the Siemens total antibody test showed 117 people with an index value of 5 or higher, 100% (**Table 2**). The

two subjects who were negative on the SDI Biosensor Rapid Antibody Test had relatively high antibody values of 49.16 U/mL and 250 U/mL on the Roche antibody test and index values of 6.27 and 10 on the Siemens Total Antibody Test.

The Roche and Siemens antibody tests were performed at 16 weeks after the first dose in 64 infection-free individuals who received two doses of the AstraZeneca vaccine, excluding 52 individuals who were lost to follow-up after the second dose. All 64 individuals tested positive on the Roche and Siemens total antibody tests (**Table 3**).

Table 2. Pfizer BNT162b2 seropositive rate and quantitative level of immunoglobin according to Elecsys COV2S and Centaur COV2T

Quantitation U/mL	Pfizer BNT162b2 – post 3 weeks			Pfizer BNT162b2 – post 16 weeks		
	Male (*n* = 40)	Female (*n* = 78)	Total (*n* = 118)	Male (*n* = 40)	Female (*n* = 78)	Total (*n* = 118)
Elecsys COV2S						
0.8–19.99	4 (10.0)	15 (19.2)	19 (16.1)	-	-	-
20–49.99	17 (42.5)	16 (20.5)	33 (28.0)	-	1 (1.3)	1 (0.9)
50–99.99	8 (20.0)	14 (17.9)	21 (18.6)	-	-	-
100–174.99	4 (10.0)	18 (23.1)	22 (18.6)	-	-	-
175–249.99	3 (7.5)	9 (11.5)	12 (10.2)	1 (2.5)	1 (1.3)	2 (1.7)
Above 250	4 (10.0)	6 (7.7)	10 (8.5)	39 (97.5)	75 (97.4)	114 (97.4)
Centaur COV2T						
Negative	1 (2.5)	1 (1.3)	2 (1.7)	-	-	-
1–4.99	14 (35.0)	28 (35.9)	42 (35.6)	-	-	-
5–10	12 (30.0)	18 (23.1)	30 (25.4)	-	1 (1.3)	1 (0.9)
> 10	13 (32.5)	31 (39.7)	44 (37.3)	40 (100.0)	76 (98.7)	116 (99.1)

Values are presented as n (%). *1 subject was not tested at post-16 weeks. Abbreviation: Centaur COV2T, Centaur XPT SARS-CoV-2; Elecsys COV2S, Elecsys Anti-SARSCoV-2 S

Table 3. AstraZeneca ChAdOx1 seropositive rate and quantitative level of immunoglobin according to Elecsys COV2S and Centaur COV2T

Quantitation U/mL	AstraZeneca ChAdOx1 – post 3 weeks			AstraZeneca ChAdOx1 – post 16 weeks		
	Male (*n* = 38)	Female (*n* = 78)	Total (*n* = 116)	Male (*n* = 38)	Female (*n* = 78)	Total (*n* = 116)
Elecsys COV2S						
Negative	2 (5.3)	3 (3.8)	5 (4.3)	-	-	-
0.8–19.99	20 (52.6)	44 (56.4)	64 (55.2)	-	-	-
20–49.99	4 (10.5)	15 (19.2)	19 (16.4)	-	-	-
50–99.99	8 (21.1)	8 (10.3)	16 (13.8)	1 (4.8)	-	1 (1.6)
100–174.99	2 (5.3)	5 (6.4)	7 (6.0)	-	-	-
175–249.99	1 (2.6)	1 (1.3)	2 (1.7)	1 (4.8)	4 (9.3)	5 (7.8)
Above 250	1 (2.6)	2 (2.6)	3 (2.6)	19 (90.5)	39 (90.7)	58 (90.6)
Centaur COV2T						
Negative	7 (18.4)	17 (21.8)	24 (20.7)	-	-	-
1–4.99	17 (44.7)	41 (52.6)	58 (50.0)	-	-	-
5–10	3 (7.9)	8 (10.3)	11 (9.5)	1 (4.8)	-	1 (1.6)
> 10	11 (28.9)	12 (15.4)	23 (19.8)	20 (95.2)	43 (100.0)	63 (98.4)

Values are presented as n (%). *52 subjects were not tested at post-16 weeks. Abbreviation: Centaur COV2T, Centaur XPT SARS-CoV-2; Elecsys COV2S, Elecsys Anti-SARSCoV-2 S

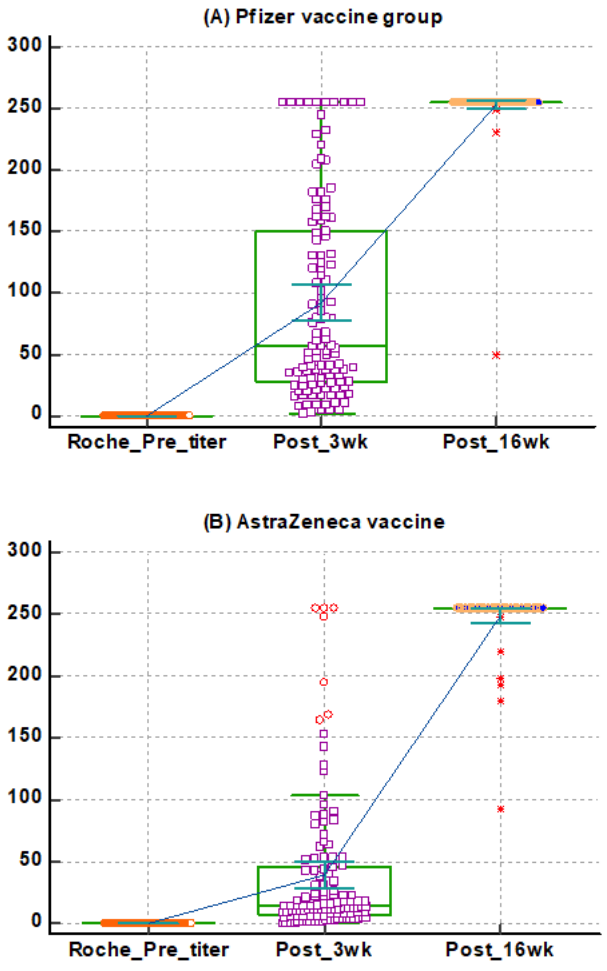

Figure 1. 3 weeks and 16 weeks post-vaccination antibody titer by vaccine groups. (A) Pfizer vaccine group; (B) AstraZeneca vaccine group.

There was no statistical difference between positive and negative Roche and Siemens total antibody test results in the Pfizer vaccine group at weeks 3 and 16 (P = 0.5, 1.0). However, the SDI Biosensor results showed a statistical difference between the Roche and Siemens results at 3 weeks after the first dose ($P < 0.0001$) and no difference at 16 weeks.

When comparing Roche antibody test results in the Pfizer and AstraZeneca groups at pre-vaccination, 3 weeks post-vaccination, and 16 weeks post-vaccination, the median antibody test level at 3 weeks in the Pfizer group was 57.1, first quartile 27.7, third quartile 150.8, and the median antibody test level at 3 weeks in the AstraZeneca group was 14.0, first quartile 6.9, third quartile 45.8. The difference between the two groups was statistically significant ($P < 0.0001$) at 3 weeks after the first dose, and there was no difference in positive results and antibody test levels at 16 weeks (P = 0.2047) (**Figure 1**). Regression analysis of Roche antibody test results and Siemens antibody test results 3 weeks after Pfizer vaccination showed a statistical association ($P < 0.0001$), as follows: Roche antibody test results (Y) = -32.9948 + 18.9242 × Siemens antibody test results (X), and Roche antibody test results (Y) = -11.5763 + 12.7533 × Siemens antibody test results (X) 3 weeks after AstraZeneca vaccination.

4. Discussion

The global spread of COVID-19 and the increasing number of deaths have become a health concern in many countries. In the absence of standard antiviral treatment guidelines for COVID-19, health authorities are deciding on pre-entry screening and quarantine periods to prevent the spread of infection between countries, and Korea has been implementing screening of infected people using real-time PCR tests for overseas arrivals and only relaxed the guidelines for screening and quarantining infected people after the Omicron infection epidemic decreased in 2022. Prior to the development of a cure for COVID-19, major pharmaceutical companies around the world were rapidly developing vaccines, both conventional adenoviral vectors and novel mRNA-based vaccines [11], and conducting licensing reviews by pharmaceutical regulatory agencies in each country to ensure rapid availability.

In the early stages of a pandemic, when sufficient vaccine production is not available, the decision of which countries to prioritize and which people to supply within a country is not only an ethical issue but also a medical and sociological rationale for finding targets where a limited number of vaccine doses will have the greatest effect in reducing the spread of infection and the number of severe illnesses or deaths [12,13]. In

general, prioritizing vaccination of high-risk groups, such as those with underlying medical conditions such as respiratory disease and those aged 65 years and over, and healthcare workers involved in the delivery of COVID-19 care, is considered to be a strategy that can preserve healthcare capacity and reduce deaths in a country.

The researchers' hospital was designated as a dedicated COVID-19 hospital, providing inpatient care for severe, moderately severe, and moderately ill patients, which allowed them to prioritize vaccination after the Ministry of Food and Drug Safety granted emergency use authorization for AstraZeneca's vaccine on 10 February 2021 and Pfizer's vaccine on 5 March 2021. Healthcare and hospital personnel who may have care or close contact with confirmed or suspected COVID-19 patients received two doses of the Pfizer vaccine 3 weeks apart, and administrative personnel with relatively little patient contact received two doses of the AstraZeneca vaccine 11 weeks apart. Subjects were enrolled in both vaccination groups in similar numbers as possible. The double representation of women in both vaccination groups is likely due to the composition of the healthcare workforce, which is dominated by nurses, with more women than men. The fact that there were only four participants aged 60 years or older was due to the fact that the study enrolment was limited to those currently working in hospitals, making it impossible to enroll people aged 70 years or older. The first and second vaccine doses were administered at 3-week intervals for the mRNA vaccine (Pfizer) and 11-week intervals for the viral vector vaccine (AstraZeneca), which are included in the 8–12 week interval, according to the recommendations of the manufacturer and the Korean Centers for Disease Control and Prevention.

A total of four people in the study tested positive for antibodies prior to vaccination, as the exclusion criteria did not include anyone who had recovered from COVID-19 infection. Since the vaccine is not contraindicated for people who have recovered from COVID-19, and the vaccine is recommended six months after recovery, it is possible to see a change in antibody levels in people who already have antibodies due to their history of infection. The Roche antibody test, which reports antibody levels above the detection range of 250 U/mL, can be diluted up to 10 times to report a maximum result of 2,500 U/mL, but this study did not perform additional dilution tests, so it can be considered a limitation that the quantitative test range was not measured high enough. Of the 238 subjects in the study, 234 were clinically determined to be free of COVID-19 antibodies, except for four individuals who had previously recovered from COVID-19 infection. Automated immunoassays, such as those from Roche and Siemens, are generally more specific than rapid antibody tests and were 100% specific in this study [14,15]. The SDI Biosensor, a rapid antibody test, had an excellent specificity of 99.1%.

One of the 45 positive SDI Biosensor rapid antibody tests measured 3 weeks after AstraZeneca vaccination was negative on both the Roche and Siemens antibody tests, suggesting a relatively large potential for false positives. However, the 16-week follow-up antibody test was missing, making it difficult to make a definitive diagnosis. The specificity of the SDI Biosensor Rapid Antibody Test for false positives was estimated to be 97.8%.

When antibody testing was performed at 3 weeks after the first dose, which is the earliest point of vaccine administration, both the Roche and Siemens automated immunoassays were able to detect antibody production early on. However, the rapid antibody test was less able to detect relatively low antibody titers than the automated immunoassay. A study by Dimeglio *et al.* reported a correlation between binding antibody titers and protective antibody titers using WHO international standards [16]. At 16 weeks, the automated immunoassay showed high antibody titers, so it is likely that neutralizing antibody titers are proportionally higher, as the actual antibodies help protect against COVID-19 infection. The standard method for testing neutralizing

antibodies is the PlaqueReduction Neutralising Test, but this method has limitations in that it cannot be automated, is time-consuming, and is labor-intensive.

The Roche automated immunoassay, which has a relatively wide reporting range of antibody titers, showed statistically significant lower antibody titers in the AstraZeneca group compared to the Pfizer group as early as 3 weeks, but no statistical significance when comparing the positive and negative groups. No statistical differences in antibody titers and positivity rates were observed at 16 weeks after the first dose of two doses each.

In conclusion, antibody formation after the first two doses of the Pfizer and AstraZeneca vaccines introduced in Korea was well established in all evaluated subjects, with antibody levels above 90% of the detectable range of the antibody test product.

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Disclosure statement

The authors declare no conflict of interest.

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