

# Quantitative Assessment of Antibodies to the SARS-CoV-2 Spike Receptor Binding Domain after COVID-19 Vaccination in People Living with HIV

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**Background:** People living with HIV (PLWH) might have less functional immunity, more associated comorbidities and be more vulnerable to have severe COVID-19 outcomes than the general population. A low immunogenicity to SARS-CoV-2 after vaccination is a concern.

**Objective:** To assess antibody level to the SARS-CoV-2 spike receptor binding domain (S-RBD) after vaccination according to the National Health Program for SARS-CoV-2 Vaccine in the HIV-infected population.

**Materials and Methods:** A cross-sectional study was conducted in the HIV-infected patients aged 18 years and over who received at least two doses of the COVID-19 vaccine between 2 and 12 weeks after last vaccination. The authors excluded participants with a history of previous SARS-CoV-2 infection within 12 weeks at enrollment. All enrolled patients were tested for antibodies to the SARS-CoV-2 S-RBD by Electro-chemiluminescence immunoassay.

**Results:** One hundred four HIV-infected participants, 62% male, 38% female, with a median age of 49 years (IQR 36 to 54.5) were enrolled. The median CD4 cell count at enrolment was 501.5 cells per  $\mu$ L (IQR 334 to 665). Most (84.62%) had undetected HIV viral load, and 41.35% had comorbidities. Eighty-nine participants received heterologous regimens including inactivated vaccines, ChAdOx1nCoV-19 vaccines and mRNA vaccines whereas 14 received homologous mRNA vaccines and only one received homologous ChAdOx1nCoV-19. All participants had detected antibodies to the SARS-CoV-2 S-RBD after vaccination. The mRNA-containing regimens had a higher antibody level than mRNA-free regimens (mean anti SARS-CoV-2 S-RBD antibodies 12,295.22 vs. 1,499.67 AU/mL,  $p=0.0025$ ).

**Conclusion:** Vaccine-induced SARS-CoV-2 S-RBD antibodies were detected in all HIV-infected participants. The antibody response appeared to be higher in the mRNA-containing regimens compared to the mRNA-free regimens.

**Keywords:** HIV; Anti SARS-CoV-2 S-RBD antibodies; COVID-19 vaccines; SARS-CoV-2

Received 6 March 2025 | Revised 26 June 2025 | Accepted 3 July 2025

J Med Assoc Thai 2025;108(Suppl.2):S145-153

Website: <http://www.jmatonline.com>

HIV infection is a major health problem in Thailand. In April 2022, the Division of AIDS and Sexually Transmitted Diseases, Department of Disease Control, Thailand, reported more than 520,000 cases of people living with HIV (PLWH). Effective antiretroviral therapy results in undetectable HIV RNA, restoration of CD4 cell counts, and favorable clinical

outcomes. Complete immune reconstitution may not be possible due to residual chronic immune activation that persists even if viral replication is successfully inhibited by antiretroviral therapy<sup>(1)</sup>. PLWH may have a suboptimal response to vaccination. A previous study of the influenza vaccine suggests that only 60% of well-controlled HIV-infected individuals without preexisting immunity to H1N1 develop protective antibody titers after immunization<sup>(2)</sup>.

PLWH are at an increased risk of fatal outcomes when admitted to the hospital for severe coronavirus disease 2019 (COVID-19) compared with HIV-negative individuals<sup>(3,4)</sup>. The World Health Organization (WHO) reported that HIV infection is a significant independent risk factor for severe/critical presentation of COVID-19 upon hospitalization and in-hospital mortality<sup>(5)</sup>. The WHO has discussed the theoretical possibility that PLWH with low CD4 cell counts may have weak immunity responses to the vaccine. However, there was no evidence to support a less robust immune response to the SARS-CoV-2 vaccine

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## How to cite this article:

Srikhajonjit S, Mootsikapun P, Foocharoen C. Quantitative Assessment of Antibodies to the SARS-CoV-2 Spike Receptor Binding Domain after COVID-19 Vaccination in People Living with HIV. J Med Assoc Thai 2025;108(Suppl.2):S145-153.

DOI: 10.35755/jmedassocthai.2025.S02.S145-S153

in this population. All PLWH should be prioritized for early vaccination, especially those with comorbidities such as chronic pulmonary disease, diabetes, hypertension, obesity, kidney disease, liver disease, Parkinson's disease, multiple sclerosis, or motor-neuron disease<sup>(6)</sup>.

Antibody levels are useful for identifying antibody responses after SARS-CoV-2 vaccination. In PLWH, there were concerns about the potential for a low immunity response after vaccination and the likelihood that this would relate to the development of severe COVID-19 disease. There were only few studies on immunogenicity of SARS-CoV-2 vaccination in PLWH<sup>(7,8)</sup>. They revealed no difference in the magnitude or persistence of SARS-CoV-2 spike-specific humoral or cellular responses.

Data on antibody responses after SARS-CoV-2 vaccination in Thai PLWH are limited. Because of the heterogeneity of vaccine regimens in Thailand, it is difficult to assume antibody responses from other previously reported studies. The authors aimed to study antibody responses after SARS-CoV-2 vaccination and factors related to antibody levels in PLWH.

This research would provide real-world data on the post-vaccination antibody (Ab) response following the Thai National Health Program for SARS-CoV-2 vaccine using anti-SARS-CoV-2 S-RBD antibody levels and enables the use of the research findings as a basis for future studies or incorporating the data into SARS-CoV-2 vaccination programs.

## Objective

The objectives of the present study are to assess the SARS-CoV-2 S-RBD Ab response after COVID-19 vaccination according to the Thai National Health Program for SARS-CoV-2 vaccine in the PLWH population and to determine factors related to levels of SARS-CoV-2 S-RBD Ab.

## Materials and Methods

### Study setting and design

This cross-sectional cohort study was performed at the infectious disease clinic, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand between April 2022 and July 2022.

The inclusion criteria consisted of 1) HIV-infected patient, 2) 18 years of age or older, 3) Receiving at least two doses of the SAR-CoV-2 vaccines, which last vaccination between 2 and 12 weeks at enrollment. The exclusion criteria consisted of 1) Previous SARS-CoV-2 infection confirmed by PCR within 12 weeks at enrollment 2) Not giving informed consent.

All eligible patients had to sign informed consent before study enrollment. Electronic and paper medical

record review included subject demographics, body mass index (BMI), co-morbidities including immunosuppressed status, HIV history (duration, antiretroviral therapy (ART), recent CD4, viral load. Vaccination data consisted of SARS-CoV-2 vaccine regimens and date of vaccination.

All enrolled patients were tested for antibodies to the SARS-CoV-2 S1-RBD levels on the same day of enrollment.

### Laboratory methods

Serum samples were evaluated for antibodies to the SARS-CoV-2 S-RBD using Elecsys SARS-CoV-2 S (Roche Diagnostics, Basel, Switzerland). The assay uses a recombinant protein representing the RBD of the S antigen in a double-antigen sandwich assay format, which favored the detection of high-affinity antibodies against SARS CoV 2. The linear range of the result was 0.4 to 250 AU/mL, and the positive cut-off level was >0.8 AU/mL, with a sensitivity of 98.8% (95% CI: 98.1 to 99.3%) and a specificity of 100% (95% CI: 99.7 to 100%)<sup>(9)</sup>. The test was compared to a VSV-based pseudo-neutralization assay in 15 clinical samples from individual patients, with a Percent Positive Agreement of 92.3% (95% CI 63.97 to 99.81%)<sup>(9,10)</sup>.

### Operational definitions

1) SARS-CoV-2 vaccine meant all approved SARS-CoV-2 vaccines in Thailand.

2) CD4 cell count and HIV viral load level meant CD4 cell count and HIV viral load data within 6 months at enrollment.

3) Antibodies to the SARS-CoV-2 spike protein receptor binding domain (RBD).

3.1) An immunoassay for the in vitro quantitative determination of antibodies (including IgG) to the SARS-CoV-2 spike (S) protein receptor binding domain (RBD) in human serum and plasma using Elecsys SARS-CoV-2 S (Roche Diagnostics, Basel, Switzerland)<sup>(9)</sup>.

3.2) Linear range 0.4 to 250 AU/mL that was further extended to 25,000 U/mL by using a 1: 100 dilutions automatically performed by the instrument.

<0.8 AU/mL=non reactive

>0.8 AU/mL=reactive

>15 AU/mL=correlated with detection of virus neutralizing effects.

4) Previous SARS-CoV-2 infection included in the present study was defined as patients who were SARS-CoV-2 positive, that was detected by polymerase chain reaction (PCR) for more than 3 months or any positive antigen rapid self-test kit (ATK).

5) Inactivated vaccines were Sinovac and Sinopharm vaccines.

6) mRNA vaccines were Pfizer BNT162b2 and Moderna mRNA-1273.

### Sample size calculation

The sample size calculation was based on a previous study by Frater et al.<sup>(7)</sup>, which found that the median anti-spike IgG level at day 56 after prime vaccination was 941 EUs. The estimated population was calculated with an alpha of 0.05, Z (0.975) of 1.96, and an error of 0.1. The required sample size was 111 subjects.

### Study outcomes

The primary outcome was the SARS-CoV-2 S-RBD Ab level after vaccination according to the Thai National Health Program for SARS-CoV-2 Vaccine in the HIV-infected population.

The secondary outcome was to determine factors related to SARS-CoV-2 S-RBD Ab levels after vaccination in HIV-infected patients.

### Statistical analysis

Demographic data were presented as percentages for categorical data and mean (range) or median with interquartile range (IQR) for continuous data. The anti-SARS-CoV-2 S-RBD antibody levels were presented as mean  $\pm$  SD or median with IQR as appropriate. The authors performed univariate analysis and one-way analysis of variance by ranks (Kruskal-Wallis Test) to determine whether there were factors associated with anti-SARS-CoV-2 S-RBD Ab levels. Linear regression was used for the identification of factors associated with the anti-SARS-CoV-2 S-RBD Ab levels. Following univariate analysis, variables with a p-value less than 0.1 were further included in a multivariable model to explore associations with participant characteristics. All statistical analyses were performed using STATA 16.0 (StataCorp., College Station, TX, USA).

### The ethical consideration

The study was designed by the authors and approved by the Human Research Ethics Committee of Khon Kaen University as per the Helsinki Declaration and the Good Clinical Practice Guidelines (HE651036). All enrolled patients were tested for Ab to the SARS-CoV-2 S-RBD levels on the same day of enrollment.

### Results

A total of 113 participants were included in the present study. Nine participants were excluded from analysis because the last dose of SARS-CoV-2 vaccine was not administered within the 2 to 12-week interval. Baseline characteristics of the 104 HIV-infected patients included in the final analysis are presented in Table 1. The median age was 49 years [interquartile range (IQR) 36, 54.5], 61.52% were male, and 41.53% had comorbidities, of which

dyslipidemia was the most common (24.04%), followed by hypertension (HT) (13.46%), and type 2 diabetes mellitus (T2DM) (8.6%). The median CD4 T-cell count was 501.5 (334, 665) cells/ $\mu$ L, and 14.42% of participants had a detectable HIV viral load within the last 6 months. All participants were on antiretroviral therapy (ART); Non-Nucleoside Reverse Transcriptase Inhibitor-Based Regimens (NNRTI-based regimen) were used by 58.65%, and integrase strand inhibitor-based regimens by 24%. Fourteen percent of participants had a history of previous SARS-CoV-2 infection, either by PCR testing more than 3 months earlier or by ATK testing at any time.

At enrollment, 8 participants received 2 doses of SARS-CoV-2 vaccine, 58 received 3 doses, 37 received 4 doses, and 1 received 5 doses. Due to the wide range of vaccination regimens (35 vaccination regimens in 104 participants) (Table 2), the authors grouped the vaccination regimens into 6 groups based on vaccine type. The Heterologous mix-and-match regimens included inactivated vaccine/ChAdOx1nCoV-19/mRNA (38.46%), ChAdOx1nCoV-19/mRNA (24%) and Inactivated vaccine/mRNA regimens (20%). Fourteen participants received homologous mRNA vaccines and only one received 3 doses of ChAdOx1nCoV-19.

All HIV-infected participants had antibody responses to SARS-CoV-2 vaccines. The median anti-SARS-CoV-2 S-RBD Ab level was 11,475 AU/mL (IQR 4,706.6, 21,835.5) in males, and 6,210.5 AU/mL (IQR 3,643, 15,564.5) in females. The median anti-SARS-CoV-2 S-RBD Ab level was 10,558 AU/mL (IQR 5,002, 17,032) in the homologous mRNA, 1,000 AU/mL (IQR 922, 1,865) in the inactivated vaccine/ChAdOx1nCoV-19, 9,577 AU/mL (IQR 5,461, 19,921) in the inactivated vaccine/mRNA, 11,739.5 AU/mL (IQR 3,398, 21,320.5) in the ChAdOx1nCoV-19/mRNA, 9,624 AU/mL (IQR 5,245, 24,175.5) in the inactivated vaccine/ChAdOx1nCoV-19/mRNA and 1,240 AU/mL in one participant who received 3 doses of the ChAdOx1nCoV-19 vaccine.

Anti-SARS-CoV-2 S-RBD Ab levels were statistically different in the inactivated/ChAdOx1nCoV-19 regimens compared to the inactivated vaccine/mRNA and inactivated vaccine/ChAdOx1nCoV-19/mRNA regimens ( $p=0.0014$  and  $0.00065$ , respectively) (Figure 1). There was no statistical difference in Anti-SARS-CoV-2 S-RBD Ab level by vaccine dose received (Figure 2).

The mRNA containing regimens (94.2%) had a higher antibody level than the mRNA-free regimens (5.8%), and a mean anti-spike RBD Ab of 12,295.22 vs. 1,499.67 AU/mL ( $p=0.0025$ ) (Table 3).

### Three-doses SARS-CoV-2 vaccine group analysis

Fifty-eight participants received 3 doses of SARS-

**Table 1.** Baseline characteristics and anti-SARS-CoV-2 S-RBD Ab levels

| Variables                                              | Total n=104            | Anti-SARS-CoV-2 S-RBD Ab levels,<br>Median, AU/mL [IQR] |
|--------------------------------------------------------|------------------------|---------------------------------------------------------|
| Age (years) median (IQR)                               | 49 (36 to 54.5)        |                                                         |
| Male, n (%)                                            | 64 (61.54)             | 11,475 (4,706.5 to 21,835.5)                            |
| Female, n (%)                                          | 40 (38.46)             | 6,210.5 (3,643 to 15,564.5)                             |
| Comorbidities, n (%)                                   | 43 (41.35)             | 8,155 (3,618 to 16,863)                                 |
| No comorbidities, n (%)                                | 61 (58.65)             | 12,180 (5,288 to 22,423)                                |
| T2DM, n (%)                                            | 9 (8.65)               |                                                         |
| HbA1c mean (range)                                     | 6.8 (4.5 to 9.8)       | -                                                       |
| HT, n (%)                                              | 14 (13.46)             |                                                         |
| HBV, n (%)                                             | 4 (3.85)               |                                                         |
| DLD, n (%)                                             | 25 (24.04)             |                                                         |
| cancer, n (%)                                          | 1 (0.96)               |                                                         |
| CD4+ cells/mL, Median (IQR)                            | 501.5 (334 to 665)     | -                                                       |
| >500 n (%)                                             | 35 (33.65)             | 10,006 (4,924 to 23,351)                                |
| 350 to 500 n (%)                                       | 16 (15.38)             | 10,184.5 (4,457.5 to 18,527.5)                          |
| 200 to 349 n (%)                                       | 13 (12.5)              | 14,725 (3,211 to 18,224)                                |
| <200 n (%)                                             | 6 (5.77)               | 1,623.5 (648 to 3,713)                                  |
| No CD4 data due to HIV VL not detected >2 years, n (%) | 34 (32.69)             | 8,632 (5,202 to 17,032)                                 |
| HIV VL, copies/ml mean (range)                         | 11,647 (20 to 157,777) | -                                                       |
| HIV VL not detected, n (%)                             | 88 (84.62)             | 9,680 (4,963 to 21,835.5)                               |
| HIV VL detected, n (%)                                 | 15 (14.42)             | 3,211 (648 to 15,390)                                   |
| SARS-CoV-2 vaccine regimens, n (%)                     |                        |                                                         |
| Homologous mRNA                                        | 14 (13.46)             | 10,558 (5,002 to 17,032)                                |
| Homologous ChAdOx1nCoV-19                              | 1 (0.96)               | 1,204                                                   |
| Inactivated vaccine/ChAdOx1nCoV-19                     | 5 (4.81)               | 1,000 (922 to 1,865)                                    |
| Inactivated vaccine/mRNA                               | 20 (19.23)             | 9,557 (5,461.5 to 19,921)                               |
| ChAdOx1nCoV-19/mRNA                                    | 24 (23.08)             | 11,739.5 (3,398 to 21,320.5)                            |
| Inactivated vaccine/ChAdOx1nCoV19/mRNA                 | 40 (38.46)             | 9,624 (5,245 to 24,175.5)                               |
| Vaccine dose received at time of study n (%)           |                        |                                                         |
| 2 doses                                                | 8 (7.69)               | 5,040 (2,095.5 to 9,073)                                |
| 3 doses                                                | 58 (55.77)             | 8,062.5 (3,735 to 16,863)                               |
| 4 doses                                                | 37 (35.58)             | 14,583 (6,422 to 23,351)                                |
| 5 doses                                                | 1 (0.96)               | 25,000                                                  |
| Previous SARS-CoV-2 infection, n (%)                   | 15 (14.4)              | 17,032 (7,970 to 25,000)                                |
| Naïve SARS-CoV-2 infection, n (%)                      | 89 (85.6)              | 8,189 (3,830 to 17,419)                                 |

IQR=interquartile range; BMI=body mass index; HT=hypertension; DM=diabetes mellitus; HBV=hepatitis B virus; DLD=dyslipidemia disease; VL=viral load

CoV-2 vaccine, the mean age was 50 years (41 to 47 years), 55% were male, 43% had comorbidities, and 15% were previously infected with SARS-CoV-2. The median CD4 count was 455 (IQR 312, 665), an undetectable HIV viral load of 87.93%, and 63% received an NNRTI-based regimen (Table 4). There were no statistical differences in anti-spike RBD Ab levels by age, BMI, smoking, comorbidity, CD4 cell count, and duration and ARV regimen.

The median levels of Anti-SARS-CoV-2 S-RBD for each vaccine regimen is shown in Table 5 and were not statistically different (Figure 3).

In univariate analysis, the factor associated with a high antibody level was previous SARS-CoV-2 infection

(p=0.002). Female sex and detectable HIV viral load were associated with a low antibody level (p=0.49 and 0.0009 respectively), (Table 4). Factors included in multivariate analysis were age, sex, comorbidities, detectable HIV viral load, and previous SARS-CoV-2 infection. Only detectable HIV viral load (p=0.031) and previous SARS-CoV-2 infection (p=0.015) were statistically significant (Table 6).

**Four-doses SARS-CoV-2 vaccine group analysis**

Baseline characteristics and Anti-SARS-CoV-2 S-RBD Ab levels in 37 participants is shown in (Table 7). Only underlying HT (3 participants) was correlated with low antibody response in both univariate and multivariate

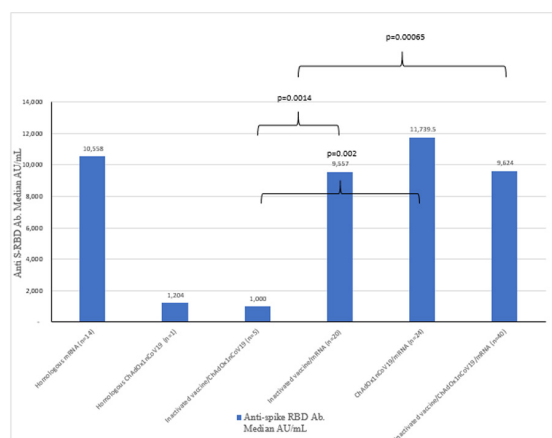
**Table 2.** SARS-CoV-2 vaccine regimens in the present study

| Regimens      | n | Regimens       | n | Regimens      | n  |
|---------------|---|----------------|---|---------------|----|
| AZ/AZ/AZ      | 1 | PZ/PZ/MDN/MDN  | 2 | SV/SV/MDN/MDN | 1  |
| AZ/AZ/MDN/MDN | 1 | PZ/MDN/MDN     | 1 | SV/SV/PZ/MDN  | 1  |
| AZ/AZ/MDN/PZ  | 2 | SP/SP/PZ       | 6 | SV/SV/PZ/PZ   | 2  |
| AZ/AZ/PZ      | 8 | SP/SP/PZ/PZ    | 4 | SV/AZ/AZ/PZ   | 1  |
| AZ/AZ/PZ/PZ   | 5 | SP/SP/PZ/MDN   | 1 | SV/AZ/AZ      | 5  |
| AZ/PZ/PZ      | 4 | SP/SP/MDN      | 2 | SV/AZ/MDN     | 3  |
| AZ/PZ/MDN/MDN | 1 | SP/SP/MDN/MDN  | 2 | SV/AZ/MDN/MDN | 2  |
| AZ/PZ         | 3 | SP/PZ/PZ       | 1 | SV/AZ/MDN/PZ  | 1  |
| MDN/MDN       | 1 | SP/AZ/PZ       | 1 | SV/AZ/PZ      | 20 |
| PZ/PZ         | 4 | SV/SV/AZ/PZ    | 3 | SV/AZ/PZ/PZ   | 1  |
| PZ/PZ/PZ      | 5 | SV/SV/AZ/PZ/PZ | 1 | SV/AZ/PZ/MDN  | 1  |
| PZ/PZ/MDN     | 1 | SV/SV/AZ/MDN   | 6 |               |    |

AZ=ChAdOx1 nCoV-19; SP=Sinopharm; PZ=Pfizer BNT162b2; MDN=Moderna mRNA-1273; SV=Sinovac

**Table 3.** Anti-SARS-CoV-2 S-RBD Ab level compared with and without mRNA

| SARS-CoV-2 vaccine regimen | n (%)     | Mean (SD)         | Mean difference (95% CI)            | p-value |
|----------------------------|-----------|-------------------|-------------------------------------|---------|
| Without mRNA               | 6 (5.8)   | 1,499.67 (1,291)  | -10,795.55 (-17,711.88, -3,879.223) | 0.0025  |
| With mRNA                  | 98 (94.2) | 12,295.22 (8,497) |                                     |         |

**Figure 1.** SARS-CoV-2 vaccines regimens and Anti-SARS-CoV-2 S-RBD Ab level.

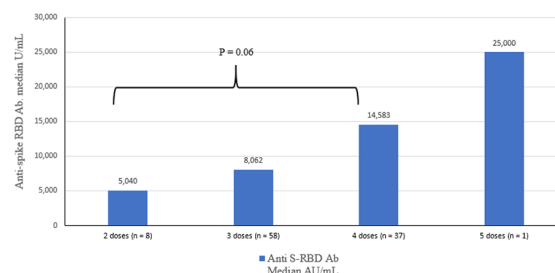
One-way analysis of variance by ranks (Kruskal-Wallis Test), multiple comparisons between groups (Adjusted p-value for significance is 0.0016).

analysis ( $p=0.32$ ,  $0.021$  respectively) (Table 8).

## Discussion

The present study showed real-world data on anti-SARS-CoV-2 S-RBD antibody levels after vaccination according to the Thai National Health Program in an HIV-infected population. There was heterogeneity in the SARS-CoV-2 vaccine regimens used in Thailand.

The primary series ranged from two doses of inactivated vaccine (Sinovac, Sinopharm), one dose of inactivated vaccine with ChAdOx1nCoV-19 or mRNA

**Figure 2.** Vaccine dose received at time of the present study.

One-way analysis of variance by ranks (Kruskal-Wallis Test), multiple comparisons between groups of (Adjusted p-value for significance is 0.0041).

vaccine (Pfizer BNT162b2, Moderna mRNA-1273), two doses of ChAdOx1nCoV-19, and two doses of mRNA. Almost all booster vaccines were mRNA vaccines (76%), followed by ChAdOx1nCoV-19 (24%).

In the present study, all participants who received the inactivated vaccine for the primary series were boosted with ChAdOx1nCoV-19 or an mRNA vaccine. Eight participants who received only a primary series all received two doses of the mRNA vaccines. All participants received ART, most had undetectable HIV viral loads (84%), and only 6 participants had CD4 cell counts below 200 cells per  $\mu\text{L}$ .

Antibodies to SARS-CoV-2 S-RBD were detected in all HIV-infected participants. These findings may indicate that after a primary series of mRNA vaccines or other primary series with a booster, HIV-infected patients receiving ART have an antibody response to SARS-CoV-2 vaccines,

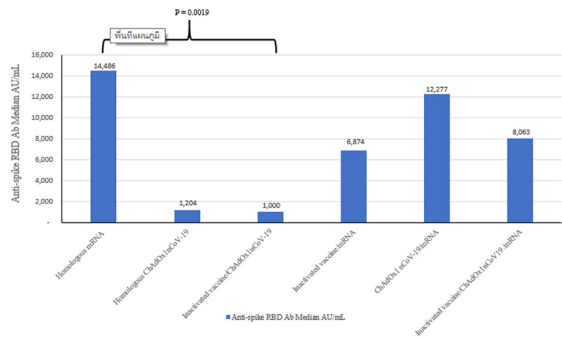
**Table 4.** Baseline characteristics, Anti-SARS-CoV-2 S-RBD Ab level and univariate analysis in 3-doses group

|                                         |                         | Anti-SARS-CoV-2 S-RBD Ab level, Median AU/mL [IQR] | Univariable models, Beta coefficient [95% CI] | p-value |
|-----------------------------------------|-------------------------|----------------------------------------------------|-----------------------------------------------|---------|
| Age (years), median (IQR)               | 50 (41 to 57)           | -                                                  | -163.48 (-333.97, 6.10)                       | 0.060   |
| Male, n (%)                             | 32 (55.17)              | 10,203.5 (4,706.5 to 22,129)                       | -4,403.62 (-8,778.49, -28.75)                 | 0.049   |
| Female, n (%)                           | 26 (44.83)              | 5,347 (2,823 to 12,373)                            |                                               |         |
| Comorbidities, n (%)                    | 25 (43.1)               | 5,202 (3,735 to 11,299)                            | -4,018.86 (-8,439.94, 402.21)                 | 0.074   |
| No comorbidities, n (%)                 | 33 (56.9)               | 10,530 (5,288 to 22,423)                           |                                               |         |
| T2DM                                    | 8 (13.79)               | 7,514.5 (4,618 to 11,836)                          | -2,237.33 (-8,744.4, 4,269.73)                | 0.494   |
| HT                                      | 11 (18.97)              | 9,108 (4,211 to 16,863)                            | -609.23 (-6,354.72, 5,136.25)                 | 0.833   |
| HBV                                     | 1 (1.72)                | 8,189 (8,189 to 8,189)                             | -2,375.18 (-19,674, 14,923.62)                | 0.784   |
| DLD                                     | 12 (20.69)              | 5,304 (3,854 to 10,125)                            | -4,281.05 (-9,724.32, 1,162.21)               | 0.121   |
| Cancer                                  | 1 (0.96)                | 1,204                                              | -                                             | -       |
| CD4+ cells/mL, median (IQR)             | 455 (312 to 665)        | -                                                  | 6.17 (-5.15, 17.50)                           | 0.276   |
| >500 n (%)                              | 18 (31.03)              | 8,344 (3,830 to 22,423)                            |                                               |         |
| 350 to 500 n (%)                        | 10 (17.24)              | 10,184.5 (4,704 to 18,776)                         |                                               |         |
| 200 to 349 n (%)                        | 6 (10.35)               | 14,081 (3,735 to 25,000)                           |                                               |         |
| <200 n (%)                              | 5 (8.62)                | 1,204 (648 to 2,043)                               |                                               |         |
| No data due to VL not detected >2 years | 19 (32.76)              | 6,874 (4,770 to 13,700)                            |                                               |         |
| HIV VL, copies/ml, mean (range)         | 2,160.43 (20 to 13,080) | -                                                  |                                               |         |
| HIV Viral load detected, n (%)          | 7 (12.07)               | 1,204 (392.8 to 2,823)                             |                                               | 0.0009  |
| HIV Viral load not detected, n (%)      | 51 (87.93)              | 8,718 (4,704 to 18,776)                            |                                               |         |
| Previous SARS-CoV-2 infection, n (%)    | 9 (15.52)               | 21,835 (13,700 to 25,000)                          | 9,063.10 (3,332.09, 14,794.12)                | 0.002   |
| Naïve SARS-CoV-2 infection              | 49 (84.48)              | 6,179 (3,456 to 12,575)                            |                                               |         |

IQR=interquartile range; BMI=body mass index; HT=hypertension; DM=diabetes mellitus; HBV=hepatitis B virus; DLD=dyslipidemia disease; VL=viral load

**Table 5.** Median levels of anti-spike RBD Ab according to vaccine regimens in 3-doses group

| SARS-CoV-2 vaccine regimens            | n (%)     | Anti-SARS-CoV-2 S-RBD A, median AU/mL [IQR] |
|----------------------------------------|-----------|---------------------------------------------|
| Homologous mRNA                        | 7 (12)    | 14,486 (3,735 to 18,776)                    |
| Homologous ChAdOx1nCoV-19              | 1 (0.96)  | 1,204                                       |
| Inactivated vaccine/ChAdOx1 nCoV-19    | 5 (8.6)   | 1,000 (922 to 1,865)                        |
| Inactivated vaccine/mRNA               | 9 (15.5)  | 6,874 (5,999 to 14,734)                     |
| ChAdOx1nCoV-19/mRNA                    | 12 (20.6) | 12,276.5 (2,953 to 24,356)                  |
| Inactivated vaccine/ChAdOx1nCoV19/mRNA | 24 (41.3) | 8,062.5 (4,986 to 16,301)                   |



**Figure 3.** Median levels of Anti-SARS-CoV-2 S-RBD Ab according to vaccine regimens in 3-doses group. One-way analysis of variance by ranks (Kruskal-Wallis Test), multiple comparisons between groups (Adjusted p-value for significance is 0.0016).

regardless of the vaccine regimens. Anti-SARS-CoV-2 S-RBD levels trended to increase with the number of booster doses, but this increase was not statistically significant.

The mRNA-containing regimens appeared to elicit a higher antibody response than the mRNA-free regimens ( $p=0.025$ ) [mean Anti-SARS-CoV-2 S-RBD Ab 12,295.22 AU/ml (SD 8,497) vs. 1,499.67 (SD 1,291)]. However, the sample size was small in the group without mRNA (6 participants). Supporting these findings, the inactivated/ChAdOx1nCoV-19 regimens had lower levels of antibody to SARS-CoV-2 S-RBD compared with the inactivated vaccine/mRNA and inactivated vaccine/ChAdOx1nCoV-19/mRNA regimens ( $p=0.0014$  and  $0.00065$ , respectively).

The authors, performed subgroup analyses by dividing

**Table 6.** Multivariate regression analysis in 3-doses group

| Variables                     | Multivariable models beta coefficient (95% CI) | p-value |
|-------------------------------|------------------------------------------------|---------|
| Age                           | -71.01 (-250.59, 108.57)                       | 0.431   |
| Sex                           | -3,628.16 (-7,685.04, 428.71)                  | 0.079   |
| Comorbidities                 | -1,792.13 (-6,410.206, 2,825.92)               | 0.440   |
| HIV viral load detected       | -6,733.98 (-12,815.88, -652.09)                | 0.031   |
| Previous SARS-CoV-2 infection | 6,955.67 (13,95.347, 12,516.01)                | 0.015   |

**Table 7.** Baseline characteristics, anti-spike RBD Ab levels and univariate analysis in 4-doses group

|                                             |                   | Anti-spike RBD Ab, median U/mL[IQR] | Univariable models, Beta coefficient [95% CI] | p-value |
|---------------------------------------------|-------------------|-------------------------------------|-----------------------------------------------|---------|
| Age (years), median (IQR)                   | 47 (35 to 54)     |                                     | -103.99 (-337.37, 129.38)                     | 0.372   |
| Male, n (%)                                 | 24 (64.86)        | 14,654 (7,535 to 23,418)            | -106.75 (-7,011.54 to 4,998.04)               | 0.736   |
| Female, n (%)                               | 13 (35.14)        | 12,534 (6,422 to 23,351)            |                                               |         |
| Comorbidities, n (%)                        | 15 (40.54)        | 10,006 (3,618 to 25,000)            | -2,021.19 (-7,828.30 to 3,785.91)             | 0.484   |
| No comorbidities, n (%)                     | 22 (59.46)        | 15,522 (6,422 to 23,351)            |                                               |         |
| T2DM                                        | 1 (2.7)           | 14,654 (6,152.5 to 24,175.5)        | -8,025.55 (-25,516.92, 9,465.82)              | 0.358   |
| HT                                          | 3 (8.11)          | 3,618 (3,040 to 6,528)              | -10,818.48 (-20,661.09, -975.88)              | 0.032   |
| HBV                                         | 1 (2.7)           | 112.8                               | -14,618.95 (-31,600.33, 2,362.42)             | 0.089   |
| DLD                                         | 11 (29.73)        | 18,279 (6,528 to 25,000)            | 2,257.249 (-3,976.98, 8,491.48)               | 0.467   |
| CD4+ cells/mL, median (IQR)                 | 559 (384 to 668)  | -                                   | 4.407391 (-14.28, 23.09)                      | 0.629   |
| >500, n (%)                                 | 13 (35.14)        | 17,419 (10,006 to 23,351)           |                                               |         |
| 350 to 500, n (%)                           | 5 (13.51)         | 12,978 (3,618 to 18,279)            |                                               |         |
| 200 to 349, n (%)                           | 5 (13.51)         | 14,725 (3,211 to 18,224)            |                                               |         |
| <200, n (%)                                 | 0                 |                                     |                                               |         |
| No data due to HIV VL not detected >2 month | 14 (37.84)        | 12,092.5 (6,422 to 25,000)          |                                               |         |
| HIV viral load not detected, n (%)          | 30 (81.08)        | 1,4583 (5,883 to 25,000)            |                                               | 1       |
| HIV viral load detected, n (%)              | 6 (16.22)         | 15,198.5 (10,006 to 18,224)         |                                               |         |
| Vaccine regimens, n (%)                     |                   |                                     |                                               |         |
| Homologous mRNA                             | 2 (5.4)           | 12,722 (5,002 to 20,442)            |                                               |         |
| Homologous ChAdOx1nCoV-19                   | 0                 | -                                   |                                               |         |
| Inactivated vaccine/ChAdOx1nCoV-19          | 0                 | -                                   |                                               |         |
| Inactivated vaccine/mRNA                    | 11 (29.7)         | 12,534 (4,924 to 25,000)            |                                               |         |
| ChAdOx1nCoV-19/mRNA                         | 9 (24.3)          | 12,978 (8,542 to 20,805)            |                                               |         |
| Inactivated vaccine/ChAdOx1nCoV19/mRNA      | 15 (40.5)         | 14,725 (5,883 to 25,000)            |                                               |         |
| Previous SARS-CoV-2 infection, n (%)        | 3 (8.11)          | 21,836 (12,978 to 25,000)           | 6,095.594 (-4,213.78, 16,404.97)              | 0.238   |
| Naïve SARS-CoV-2 infection n (%)            | 34 (91.89)        | 13,558.5 (5,883 to 2,3351)          |                                               |         |
| HIV VL mean (range)                         | 292 (25 to 1,453) |                                     |                                               |         |

IQR=interquartile range; BMI=body mass index; HT=hypertension; DM=diabetes mellitus; HBV=hepatitis B virus; DLD=dyslipidemia disease; VL=viral load

**Table 8.** Multivariate analysis in 4-doses group

|     | Multivariable models Beta coefficient [95% CI] | p-value |
|-----|------------------------------------------------|---------|
| HT  | -11,276.09 (-20,752.57, -1,799.61)             | 0.021   |
| HBV | -15,558.62 (-3,1509.91, 392.66)                | 0.056   |

HT=hypertension; HBV=hepatitis B virus

the participants into 3-dose and 4-dose vaccine groups. In all subgroup analyses, the vaccine regimen was not correlated with anti-SARS-CoV-2 S-RBD levels. Data suggested that a history of previous SARS-CoV-2 infection was associated with a higher antibody response in the 3-dose group, but

this association was not seen in the 4-dose vaccine group. It could be interpreted that the fourth booster dose with an mRNA vaccine might overcome the antibody responses from previous SARS-CoV-2 infections. However, given the small samples of previous SARS-CoV-2 infections in each cohort (15% in the 3-dose group and 8% in the 4-dose vaccination group), the results should be interpreted with caution. A similar result was observed with detectable HIV viral load, which was associated with low antibody levels in the 3-dose group, but this effect was not observed in the 4-dose vaccine group.

In the 4-dose subgroup analysis, we, the authors, found that hypertension was associated with low antibody levels ( $p=0.021$ ). However, there were only three participants with hypertension in this group, which makes it difficult to explain these findings due to the small sample size. Hypertension was not statistically significantly associated with anti-SARS-CoV-2 S-RBD levels among the other subgroups. Further research is needed to evaluate these findings.

Frater et al. reported the safety and immunogenicity of the ChAdOx1 CoV-19 vaccine compared between HIV-infected participants aged 18 to 55 years ( $CD4 >350$  cells/mL, viral load  $<50$  copies per mL, on ART) and those without HIV. In the HIV group, anti-spike IgG responses by ELISA peaked at day 42 (median 1,440 ELISA units [EUs; IQR 704 to 2,728],  $n=50$ ) which were sustained until day 56 (median 941 EUs [531 to 1,445],  $n=49$ ). Compared with participants without HIV, there was no difference in the magnitude or persistence of SARS-CoV-2 spike-specific humoral or cellular responses ( $p>0.05$  for all analyses). There was no correlation between the magnitude of the anti-spike IgG response at day 56 and CD4 cell counts ( $p=0.93$ ) or age ( $p=0.48$ )<sup>(7)</sup>.

Feng et al. reported the immunogenicity after two doses of BBIBP-CoV vaccines in 42 PLWH and 28 healthy individuals. They reported a similarity between the binding antibody, neutralizing antibody, and S protein specific T cell responses in PLWH and healthy individuals. However, although PLWH with low baseline  $CD4^+/CD8^+$  T cell ratios ( $<0.6$ ) generated a lower antibody response than PLWH with medium (0.6 to 1.0) or high ( $>1.0$ ) baseline  $CD4^+/CD8^+$  T cell ratios ( $p<0.01$ )<sup>(8)</sup>.

A recent systematic review of COVID-19 vaccine antibody responses in people with HIV<sup>(11)</sup>, which included 28 studies from 12 countries from 1 January 2020 to 31 March 2022, reported high COVID-19 vaccine seroconversion rates in PLWH. PLWH with lower baseline CD4 counts,  $CD4/CD8$  ratios, or higher baseline viral loads had lower seroconversion rates and immunologic titers. Global COVID-19 vaccine shortages may limit homologous vaccine use, so heterologous mix-and-match strategies require evaluation for maximum immunogenicity and effectiveness against challenging variants of concern and other subvariants, optimized dosing intervals, and boosting strategies.

Our findings were similar to the results of this systematic review. After SARS-CoV-2 vaccination, all HIV-infected participants had seroconversion. Detectable HIV viral load appeared to correlate with a low antibody response. In addition, heterologous mix-and-match SARS-CoV-2 vaccine strategies also had a high seroconversion rate. We, the authors, believe this information is useful in

countries that use mix-and-match SARS-CoV-2 vaccine strategies.

CD4 T cells are reported to be associated with both humoral and cellular immune responses to COVID-19 vaccination, and play a pivotal role in the vaccination management. Regulatory T cells (Tregs) are one of CD4 subsets with the ability to maintain immune homeostasis in COVID-19. Many studies revealed low anti-SARS-CoV-2 S-RBD Ab levels in PLWH with CD4 T cells 200 to 500/mm<sup>3</sup>, whereas robust response in PLWH with CD4 T cells  $>500$ /mm<sup>3</sup> comparable to HIV-negative population<sup>(11-14)</sup>. However, in the present study there was no statistical difference in anti-SARS-CoV-2 S-RBD Ab levels in each CD4 group. The lack of significant differences in antibody levels across CD4 subgroups following COVID-19 vaccination could be explained by several factors. While CD4<sup>+</sup> T cells play a crucial role in immune responses, the antibody response itself may not be as directly impacted by CD4 subtype differences. The robust nature of the vaccine response and the potential contributions of other immune cell populations may obscure the specific impact of CD4 count variations on the antibody level response in COVID-19 vaccination.

There were several limitations in our study. First, the heterogeneity in vaccine regimens made it difficult to detect other factors that correlate with anti-SARS-CoV-2 S-RBD Ab levels, so we, the authors, attempted to group vaccine regimens for analysis. Second, the population with low CD4 counts accounted for only a small proportion of the study (5%, CD4 cell count  $<200$ /mm<sup>3</sup>). The result of lack of significance in antibody response to the other CD4 subgroup should be cautiously interpreted. Third, data on previous SARS-CoV-2 infections were participant reports, without laboratory confirmation. Unrecognized SARS-CoV-2 infection might have gone unidentified. Fourth, due to the heterogeneity of vaccine regimens, it was not possible to find a control group for comparison. Finally, this was a single-center study, so the results might not be generalizable.

However, the strengths of the present study were that it provided real-world data on anti-SARS-CoV-2 S-RBD Ab levels after vaccination in the HIV-infected population, and we, the authors, attempted to determine factors related to anti-SARS-CoV-2 S-RBD Ab levels. Therefore, the findings might be applied for evaluating anti-SARS-CoV-2 S-RBD Ab levels among the population who had heterogeneous SARS-CoV-2 vaccine regimens.

## Conclusion

All HIV-infected participants had an antibody response after SARS-CoV-2 vaccination (seroconversion) regardless of vaccine regimens. The antibody levels appeared to be higher in the mRNA-containing regimens compared to the

mRNA-free regimens. Previous SARS-CoV-2 infection was associated with a high antibody level, and detectable HIV viral load was associated with a low antibody level in participants receiving 3 doses of the SARS-CoV-2 vaccine.

### What is already known on this topic?

HIV infection is a significant independent risk factor for severe/critical presentation of COVID-19 upon hospitalization and is associated with higher in-hospital mortality. HIV-infected patients with low CD4 cell counts may have weak immune responses to vaccination.

### What this study adds?

After a primary series of mRNA vaccines or other primary series with a booster, HIV-infected patients receiving ART have an antibody response to SARS-CoV-2 vaccines regardless of the vaccine regimens. Anti-SARS-CoV-2 S-RBD levels trended to increase with the number of booster doses, although this trend was not statistically significant. Detectable HIV viral load appeared to correlate with a low antibody response. In addition, heterologous mix-and-match SARS-CoV-2 vaccine strategies also resulted in a high seroconversion rate.

### Acknowledgements

The authors express gratitude to the Department of Medicine, Infectious Diseases faculty at Srinagarind Hospital, laboratory personnel, out-patient nurses at Srinagarind Hospital, statisticians, and all participants who were involved in this study.

### Funding statement

The study received funding support from the Research and Graduate Studies, Khon Kaen University, Thailand.

### Conflicts of interest

The authors declare no conflict of interest.

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