HUMAN VACCINES & IMMUNOTHERAPEUTICS
2025, VOL. 21, NO. 1, 2501467
https://doi.org/10.1080/21645515.2025.2501467

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RESEARCH ARTICLE

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Safety and immunogenicity of SARS-CoV-2 protein subunit recombinant vaccine (Indovac®) in healthy populations aged 18 years and above in Indonesia: A phase I, observer-blind, randomized, controlled study

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ABSTRACT

Indonesian vaccine producer (PT BIOFARMA), conducted a study to assess the safety and immunogenicity of a new COVID-19 vaccine candidate. This vaccine is based on a recombinant subunit protein platform, with the SARS-CoV-2 receptor-binding domain (RBD) as its target antigen. The study compared the candidate's safety and immunogenicity to the control group vaccine, the Sinovac vaccine, 28 days after administration. This was an observer-blinded and randomized Phase 1 trial which recruited 175 subjects. The subject received 0.5 ml of vaccine in two doses. The subjects were split into five treatment groups, consisting of different combinations of doses between RBD and CpG. The safety of this vaccine was evaluated within 7 days after the first dose and for 6 months after the second dose, while the immunogenicity was evaluated on days 14 and 28 after the second dose. The overall incidence of AEs was 54.86% from the beginning of the vaccination to 28 days after each injection. Most AEs were local pain and had no serious AEs. The study revealed a significant rise in the Geometric Mean Titer (GMT) of IgG antibodies in every group, indicating a strong immune response. The phase I data demonstrated that the new vaccine candidate showed promising results in safety and immunogenicity.

ARTICLE HISTORY
Received 5 September 2024
Revised 14 April 2025
Accepted 30 April 2025

KEYWORDS
COVID-19 vaccine; Indovac; safety; phase-1; immunogenicity

Introduction

COVID-19 has claimed many lives since its initial emergence as a severe acute respiratory disease in Wuhan, China.[1,2] The causative agent, SARS-CoV-2, rapidly mutated, leading to the emergence of numerous variants, with the Delta variant causing the highest number of fatalities worldwide, including in Indonesia.[1–3–5] SARS-CoV-2 belongs to the *Coronaviridae* family, which is classified into four genera: α, β, γ, and δ coronaviruses.[6] Among these, β-coronaviruses are the primary group known to infect humans.[6] This genus is further subdivided into lineages A through D, with SARS-CoV and SARS-CoV-2 both belonging to lineage B.[7,8]

SARS-CoV-2 contains four major structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N). The spike protein plays a crucial role in viral entry and consists of two functional subunits: S1 and S2.[9] The S1 subunit contains the receptor-binding domain (RBD), which is responsible for binding to the host cell receptor ACE2, while the S2 subunit mediates membrane fusion. The S1 subunit is considered the immunodominant antigen during coronavirus infections and induces long-lasting, broad-spectrum neutralizing antibody and T-cell immune responses.[10,11]

Multiple vaccines have been developed against SARS-CoV-2, utilizing various platforms including mRNA-based, viral vector, whole-pathogen inactivated virus, and subunit vaccines containing fragments of the virus.[12] The unprecedented scale of the COVID-19 pandemic prompted urgent global efforts to develop and distribute vaccines. However, significant inequities emerged during the first year of vaccine distribution, especially in low- and middle-income countries. As the world's fourth most populous country, Indonesia aimed to vaccinate at least 70% of its population – approximately 181 million people – by the end of 2021.[13] Despite the Emergency Use Authorization (EUA) of 23 vaccines globally and the authorization of 10 vaccines in Indonesia by November 2, 2021; vaccination targets remained unmet due to challenges in global distribution and limited access to adequate supplies.[14] To

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/21645515.2025.2501467

address these issues, Indonesia invested in developing its vaccines to stabilize the supply of safe, affordable vaccines and reduce reliance on foreign production. PT Bio Farma, an Indonesian vaccine producer with expertise in fermentation technology using *Pichia pastoris*, began developing a vaccine candidate based on recombinant protein subunit technology. This vaccine combines receptor-binding domain (RBD) antigens with CpG 1018 and aluminum hydroxide adjuvants, designed to stimulate antibody production capable of neutralizing the wild-type SARS-CoV-2 virus.[15] This study aimed to evaluate the safety and immunogenicity of the recombinant protein subunit vaccine and to compare the incidence of adverse events between the vaccine and control groups. Although the immediate crisis of COVID-19 has subsided, the findings of this study would contribute to the body of evidence on protein subunit-based vaccines and support Indonesia's efforts to meet its vaccination needs.

Materials and methods

This trial was an observer-blinded, comparative, and randomized phase I study, with 175 participants included. The vaccine candidate, the SARS-CoV-2 protein subunit recombinant vaccine (Indovac), was compared to a control (Sinovac vaccine). The trial utilized four different formulations of the vaccination candidate. The administration protocol for the experimental product involves injecting 0.5 ml in two separate doses, with a 28-day interval between each dosage.

The study was conducted from 2022 until 2023 by Faculty of Medicine, Universitas Diponegoro, Semarang, and Faculty of Medicine, Universitas Indonesia. The ethical approval was granted by the health research ethics committee of the Faculty of Medicine, Universitas Diponegoro (24/EC/KEPK/FK-UNDIP/I/2022) and Faculty of Medicine, Universitas Indonesia (KET-121/UN2.F1/ETIK/PPM.00.02/2022). Indonesian National Agency for Drug and Food Control approved the clinical trial (B.R.G .0106.1.1.02.22.22) before the commencement of the study. This clinical trial has also been registered on clinicaltrial.gov (NCT05228613).

The blinded investigator team checked inclusion and exclusion criteria. This clinical trial included clinically healthy individuals aged 18–70 years who were properly informed about the study, signed the informed consent and agreed to comply with trial instructions and schedules. Participants were excluded if they were enrolled in another trial, had a COVID-19 infection or vaccination history, or tested positive for SARS-CoV-2. Other exclusions included current illness, pregnancy, lactation, recent vaccinations, uncontrolled chronic diseases, immunosuppressive conditions, allergies to vaccines, or any condition that could interfere with the trial's objectives. Additionally, those planning to relocate during the study period were not eligible.

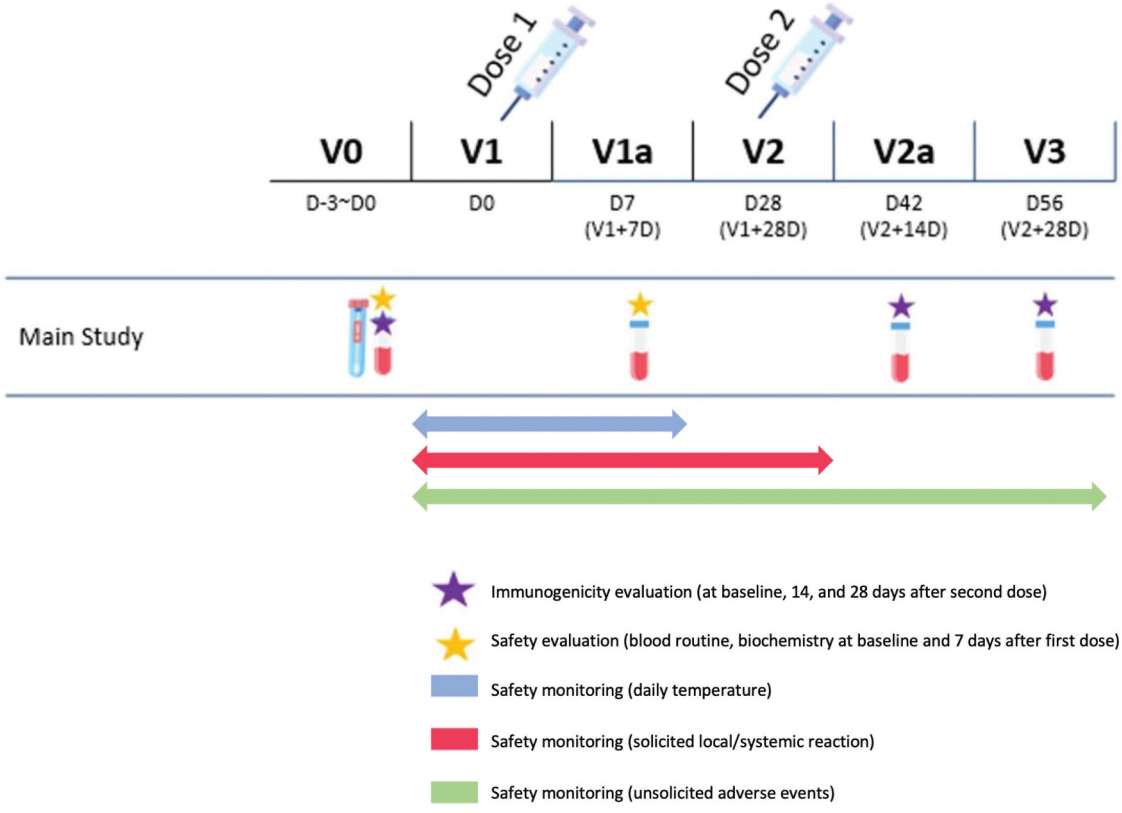
The unblinded investigator team allocated the inclusion number and randomization code (A/B/C/D) and vaccinated each recruited subject. The randomization code was kept confidential and not opened until the study's end. Each participant was given an inclusion number (ranging from 001 to 175) and a randomization code (A, B, C, or D,). Formula A (RLCL) contained 12.5 µg of SARS-CoV-2 RBD subunit recombinant protein, 750 µg of aluminum as an adjuvant, 750 µg of CpG 1018 as an adjuvant, 2.226 mg of NaCl, and 0.923 mg of tris-(hydroxymethyl)aminomethane. Formula B (RLCH) contained 12.5 µg of SARS-CoV-2 RBD subunit recombinant protein, 750 µg of aluminum as an adjuvant, 1500 µg of CpG 1018 as an adjuvant, 2.204 mg of NaCl, and 0.914 mg of tris-(hydroxymethyl)aminomethane. Formula C (RHCL) contained 25 µg of SARS-CoV-2 RBD subunit recombinant protein, 750 µg of aluminum as an adjuvant, 750 µg of CpG 1018 as an adjuvant, 2.226 mg of NaCl, and 0.923 mg of tris-(hydroxymethyl)aminomethane. Formula D (RHCH) contained 25 µg of SARS-CoV-2 RBD subunit recombinant protein, 750 µg of aluminum as an adjuvant, 1500 µg of CpG 1018 as an adjuvant, 2.204 mg of NaCl, and 0.914 mg of tris-(hydroxymethyl)aminomethane. The administration protocol for the experimental product involves injecting 0.5 ml in two separate doses, with a 28-day interval between each dosage. Immunogenicity evaluation was carried out using antibody testing after each vaccine dosage.

Safety measurements

We evaluated the severity, duration, and correlation of each adverse event. The study assessed both local and systemic reactions that occurred within 30 min to 28 days after each vaccination. These reactions were analyzed by conducting interviews with the subjects during the post-surveillance visits (V2, V3). The body temperature was measured for 7 days following vaccination. The highest temperature registered during this period was noted in the diary card, expressed in Celsius degrees, using a thermometer. The study assessed the major adverse effects 6 months after the previous immunization. The trial team documented the material in the Case Report Form (CRF). Baseline (V0), 7 days after the first dosage (V1a), and 14 days after the second dose (V2a), routine biochemical and hematological tests, urine analysis, and ECG are conducted for safety evaluation. All subjects included in the study were analyzed with intention to treat analysis.

Immunogenicity measurement

The blood samples were collected from all individuals three times, i.e., at the beginning of the study, 14 days, and 28 days after the second dose of vaccination (Figure 1) to evaluate COVID-19-specific neutralizing and isotype-specific (IgG) antibodies, seropositive rate, geometric mean titer (GMT), and seroconversion rate. GMT was analyzed using GraphPad Prism 93.0. Data were calculated after log-transformation and represented as the geometric mean titer (GMT) and 95% confidence interval (95% CI). The chemiluminescent microparticle immunoassay/CMIA (Architect$^{(R)}$, Abbott Laboratories, Illinois, USA) was used to measure the anti-SARS-CoV-2 RBD IgG antibodies. Seropositive was defined as having a titer of at least 7.1 BAU/mL. Seroconversion was defined as at least four-fold increase in anti-RBD antibody IgG titer compared to the initial level. Neutralizing antibody



Treatment Groups	Vaccine Components
Group A (RLCL)	12.5 μg of SARS-CoV-2 RBD subunit + 750 μg of CpG 1018 + 750 μg of aluminum
Group B (RLCH)	12.5 μg of SARS-CoV-2 RBD subunit + 1500 μg of CpG 1018 + 750 μg of aluminum
Group C (RHCL)	25 μg of SARS-CoV-2 RBD subunit + 750 μg of CpG 1018 + 750 μg of aluminum
Group D (RHCH)	25 μg of SARS-CoV-2 RBD subunit + 1500 μg of CpG 1018 + 750 μg of aluminum

Figure 1. Study design.

(Nab) titers were assessed by the National Health Research & Development Agency (Balitbangkes)/Litbangkes & Bio Farma using a modified cytopathogenic impact test with wild-type virus. This virus (Wuhan and Delta strain) was obtained from clinical samples and propagated in-house using Vero SLAM cells. Inactivated serum was added to the well plates and serially diluted. Subsequently, a specific amount of wild-type virus was added. Susceptible Vero SLAM cells in culture were then introduced to the mixture. After a 5–7-day incubation period, the cells were examined for signs of viral infection, specifically cytopathic effect (CPE).[16] A titer of 1:4 or greater indicated seropositivity. A titer of 1:4 or greater indicated seropositivity. All these measurements were also performed for the Wuhan and Delta strains of SARS-CoV-2. Specimens with high titers, above 1:768, were retested to determine the final titer. Fisher's test was used to compare the proportion of participants with seropositive and seroconversion between vaccinated group and control group. Kruskal–Wallis rank test was used to compare the GMT between vaccinated group and control group.

Results

In this phase I study, 175 subjects are involved, and each group in the main study consists of 35 subjects. The participant enrollment and disposition throughout the study are summarized in Figure 2.

Demographic characteristics

The demographic data is shown in Table 1 below. The male was predominant, 130 (74.29%) of the total. The average age of the participants was 36.35 ± 13.64 years. Most participants had completed junior high school (32.00%) and were employed (72.57%). The participants represented a diverse range of ethnicities, with the majority being Javanese (61.71%), followed

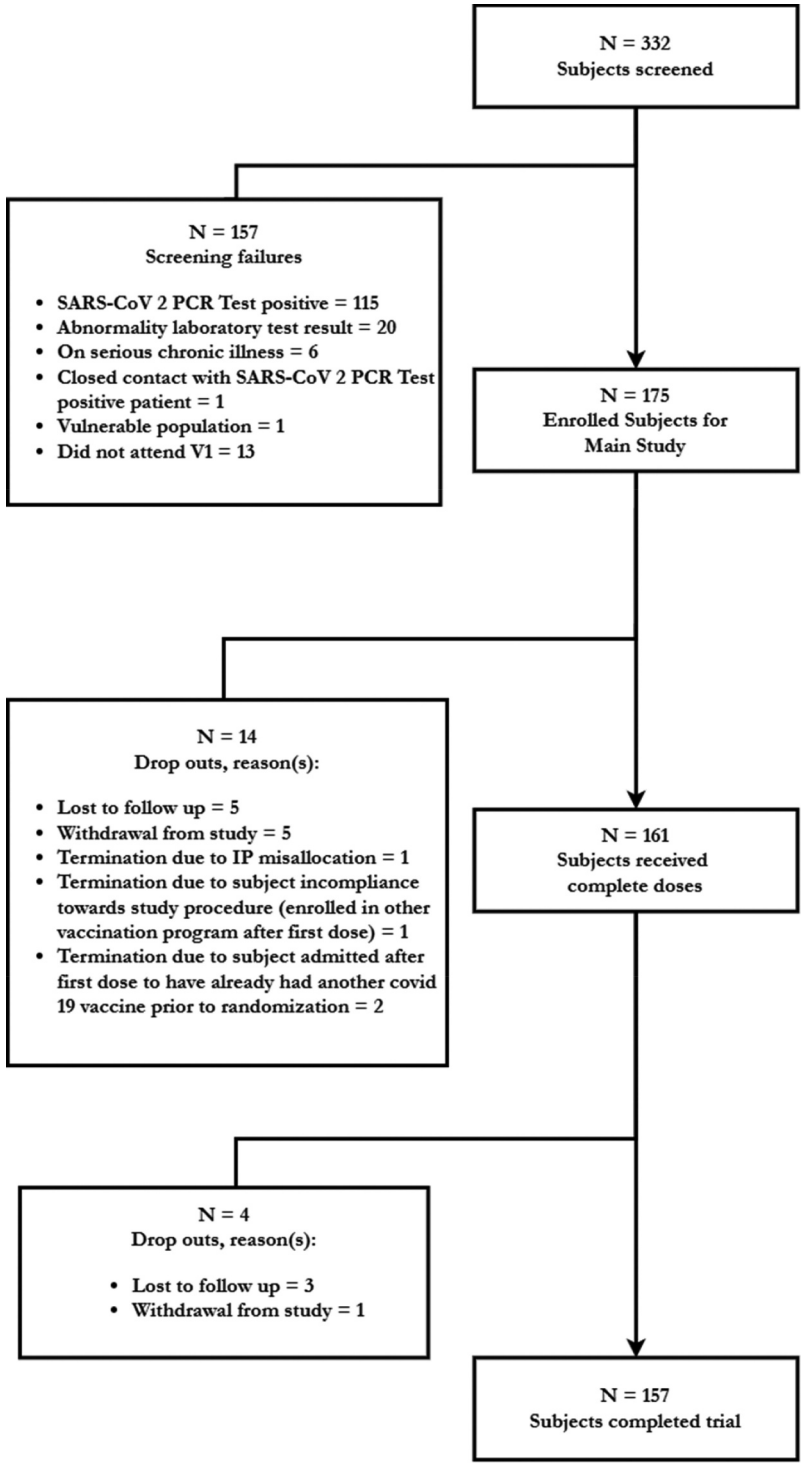


Figure 2. Subjects disposition.

by Sundanese (24.57%), Betawi (12.57%), Ambonese (0.57%), and Batak (0.57%).

Safety evaluation

In this study, the subjects were vaccinated with two doses of Indovac vaccine or Sinovac Vaccine at 28-day intervals of the immunization schedule. All participants were included in the safety population (ITT). The safety data set in this report consists of solicited and unsolicited adverse events collected at 30 min, 0–7 days, and >7 days after the first and second injections. Out of 175 participants, 96 (54.86%) experienced at least one AE. The RHCL group reported the highest proportion of participants with AEs (24/35, 68.57%), while the RLCL group had the lowest (15/35, 42.86%). However, the differences in overall AE incidence among groups were not statistically significant ($p = .148$) (Table 2).

A detailed comparison of solicited local and systemic AEs following both doses is shown in Table 3. Local reactions were most frequently reported as pain at the injection site, which

Table 1. Demographic and characteristics.

Parameter	Sinovac *N* = 35	RLCL/A *N* = 35	RLCH/B *N* = 35	RHCL/C *N* = 35	RHCH/D *N* = 35	Total *N* = 175
Mean age, [years] (SD)	34.69 (12.48)	34.60 (13.39)	34.06 (12.82)	36.49 (15.05)	41.94 (13.50)	36.35 (13.64)
Mean height, [m] (SD)	162.77 (61.81)	161.98 (77.12)	163.75 (75.46)	160.59 (72.49)	161.31 (81.43)	162.08 (73.93)
Mean weight, [kg] (SD)	55.03 (9.54)	62.78 (12.96)	59.36 (11.84)	57.70 (12.12)	59.39 (9.05)	58.85 (11.36)
BMI, (kg/m2)	20.70 (2.85)	23.94 (4.80)	22.12 (3.90)	22.39 (4.52)	22.84 (3.25)	22.40 (4.03)
Sex, n (%)						
Male	28(80.00)	22 (62.86)	29 (82.86)	27 (77.14)	24 (68.57)	130 (74.29)
Female	7 (20.00)	13 (37.14)	6 (17.14)	8 (22.86)	11 (31.43)	45 (25.71)
Education, n (%)						
No formal school	2 (5.71)	3 (8.57)	6 (17.14)	6 (17.14)	4 (11.43)	21 (12.00)
Primary school	8 (22.86)	8 (22.86)	5 (14.29)	6 (17.14)	12 (34.29)	39 (22.29)
Junior high school	14 (40.00)	10 (28.57)	11 (31.43)	9 (25.71)	12 (34.29)	56 (32.00)
Senior high school	10 (28.57)	12 (34.29)	10 (28.57)	12 (34.29)	7 (20.00)	51 (29.14)
College or university (diploma)	0 (0.00)	2 (5.71)	0 (0.00)	1 (2.86)	0 (0.00)	3 (1.71)
College or university (bachelor)	0 (0.00)	0 (0.00)	1 (2.68)	0 (0.00)	0 (0.00)	1 (0.57)
Master's or doctoral degree	1 (2.86)	0 (0.00)	2 (5.71)	1 (2.86)	0 (0.00)	4 (2.29)
Employment, n (%)						
Yes	25 (71.43)	21 (60.00)	26 (74.29)	27 (77.14)	28 (80.00)	127 (72.57)
State employee/military/police officer	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Private employee	4 (11.43)	4 (11.43)	3 (8.57)	4 (11.43)	5 (14.29)	20 (11.43)
Entrepreneur	5 (14.29)	7 (20.00)	8 (22.86)	12 (34.29)	7 (20.00)	39 (22.29)
Labor	15 (42.86)	9 (25.71)	13 (37.14)	11 (31.43)	16 (45.71)	64 (36.57)
Others	1 (2.86)	1 (2.86)	2 (5.71)	0 (0.00)	0 (0.00)	4 (2.29)
No	10 (28.57)	14 (40.00)	9 (25.71)	8 (22.86)	7 (20.00)	48 (27.43)
Ethnicity, n (%)						
Ambonese	0 (0.00)	0 (0.00)	1 (2.86)	0 (0.00)	0 (0.00)	1 (0.57)
Batak	1(2.86)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.57)
Betawi	5 (14.29)	2 (5.71)	5 (14.29)	5 (14.29)	5 (14.29)	22 (12.57)
Javanese	21 (60.00)	23 (65.71)	22 (62.86)	21 (60.00)	21 (60.00)	108 (61.71)
Sundanese	8 (22.86)	10 (28.57)	7 (20.00)	9 (25.71)	9 (25.71)	43 (24.57)

occurred in 52 participants (29.71%), followed by redness, induration, and swelling. Systemic solicited AEs included myalgia (14.86%), fatigue (13.14%), and fever (4.57%). The RHCL group showed the highest frequencies of both local and systemic solicited AEs, although differences between groups were not statistically significant.

Immunogenicity

This study measured antibody titers following dose administration at baseline, 14 and 28 days post-second dose using IgG, and surrogate Virus Neutralization Test (sVNT), for 175 subjects.

Antibody IgG titer

On the 14th and 28th days after second injection, the seropositive rate of the RLCL/A, RLCH/B, and the control Sinovac group are 100%, showed no significant difference with between groups. The RHCH/D group showed highest seroconversion 4-fold increase antibody on 14th and 28th days (88.89%), followed by the RLCH/B group (81.25%), but are not significantly different with the other three groups. The highest GMT (BAU/mL) is shown by RLCH/B group in both 14th and 28th days, which is significantly higher among the others (Figure 3 and Table S1 (Suppl. Data)).

sVNT

The baseline data on seropositive rate showed no difference among groups. On 14th and 28th days after second dose, the seroconversion 4-fold increase of antibody of group RLCH/B and RHCH/D groups are the highest among others, but with no significant difference. The GMT antibody neutralization (sVNT) of group RLCH/B on 14th and 28th days are significantly higher among groups as showed in Figure 4 and Table S2 (Suppl. Data).

Neutralizing antibody titer (against Wuhan and Delta strain)

Microneutralization (MNT)

The data were analyzed as follows: 1) GMT is the comparison result after log-transformation, 2) 95% CI, 3) Seropositive was defined as titer ≥ 46.03 IU/mL, 4) Seroconversion was defined as a change from seronegative to seropositive; or a 4-fold increase from baseline titers. The neutralization antibody assay was conducted against the Wuhan strain (Figure 5) and Delta strain (Figure 6).

Wuhan strain. Before vaccination, baseline neutralizing antibody titers were relatively low across all groups, with the Sinovac group showing the lowest GMT (62.44 IU/mL) and the RHCL group the highest (203.69 IU/mL). Seropositive rates at this time point ranged from 44% in the Sinovac group to 75% in the RLCH group; however, these differences

Table 2. Overall incidence of adverse events.

	Sinovac (N = 35)		RLCL/A (N = 35)		RLCH/B (N = 35)		RHCL/C (N = 35)		RHCH/D (N = 35)		Total (N = 175)		
Adverse Events	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	*p*-value[a]
Overall adverse events	46	19 (54.29)	44	15 (42.86)	57	22 (62.86)	59	24 (68.57)	52	16 (45.71)	258	96 (54.86)	0.148
Local	13	9 (25.71)	13	9 (25.71)	25	14 (40.00)	26	16 (45.71)	23	11 (31.43)	100	59 (33.71)	0.291
Systemic	33	17 (48.57)	31	13 (37.14)	32	18 (51.43)	33	20 (57.14)	29	14 (40.00)	158	82 (46.86)	0.432
Solicited	24	14 (40.00)	22	11 (31.43)	43	16 (45.71)	38	18 (51.43)	31	14 (40.00)	158	73 (41.71)	0.526
Local	13	9 (25.71)	13	9 (25.71)	25	14 (40.00)	26	16 (45.71)	22	11 (31.43)	99	59 (33.71)	0.291
Systemic	11	8 (22.86)	9	7 (20.00)	18	11 (31.43)	12	8 (22.86)	9	8 (22.86)	59	42 (24.00)	0.837
Unsolicited	22	13 (37.14)	22	9 (25.71)	14	11 (31.43)	21	16 (45.71)	21	11 (31.43)	100	60 (34.29)	0.470
Local	0	0 (0.00)	0	0 (0.00)	0	0 (0.00)	0	0 (0.00)	1	1 (2.86)	1	1 (0.57)	
Systemic	22	13 (37.14)	22	9 (25.71)	14	11 (31.43)	21	16 (45.71)	20	11 (31.43)	99	60 (34.29)	0.470
Within 30 mins	8	5 (14.29)	3	3 (8.57)	16	8 (22.86)	13	7 (20.00)	5	5 (14.29)	45	28 (16.00)	0.520
0–7 days	32	15 (42.86)	35	14 (40.00)	34	16 (45.71)	31	16 (45.71)	41	13 (37.14)	173	74 (42.29)	0.940
>7 days	6	5 (14.29)	6	5 (14.29)	7	7 (20.00)	15	11 (31.43)	6	5 (14.29)	40	33 (18.86)	0.279

Solicited local adverse event: pain, redness, induration, and swelling; solicited systemic adverse events: fever, fatigue, myalgia [a])Analyzed using Chi-square.

Table 3. Comparison of overall solicited AEs between groups (First and second injection).

	Sinovac N = 35		RLCL N = 35		RLCH N = 35		RHCL N = 35		RHCH N = 35		Total N = 175	
Adverse Events	No. of events	No. of subject n (%)	No. of events	No. of subjects n (%)	No. of events	No. of subjects n (%)	No. of events	No. of subjects n (%)	No. of events	No. of subjects n (%)	No. of events	No. of subjects n (%)
Local reactions	13	9 (25.71)	13	9 (25.71)	25	14 (40.00)	26	16 (45.71)	22	11 (31.43)	99	59 (33.71)
Local pain	7	7 (20.00)	11	8 (22.86)	16	13 (37.14)	17	13 (37.14)	15	11 (31.43)	66	52 (29.71)
Redness	3	3 (8.57)	0	0 (0.00)	3	3 (8.57)	2	1 (2.86)	3	3 (8.57)	11	10 (5.71)
Induration	2	2 (5.71)	0	0 (0.00)	2	2 (5.71)	4	3 (8.57)	3	3 (8.57)	11	10 (5.71)
Swelling	1	1 (2.86)	2	2 (5.71)	4	4 (11.43)	3	3 (8.57)	1	1 (2.86)	11	11 (6.29)
Systemic events	11	8 (22.86)	8	7 (20.00)	18	11 (31.43)	12	8 (22.86)	9	8 (22.86)	58	42 (24.00)
Fever	1	1 (2.86)	2	1 (2.86)	4	4 (11.43)	2	2 (5.71)	0	0 (0.00)	8	8 (4.57)
Fatigue	7	7 (20.00)	1	1 (2.86)	8	8 (22.86)	2	2 (5.71)	5	5 (14.29)	23	23 (13.14)
Myalgia	3	3 (8.57)	6	6 (17.14)	6	6 (17.14)	8	7 (20.00)	4	4 (11.43)	27	26 (14.86)

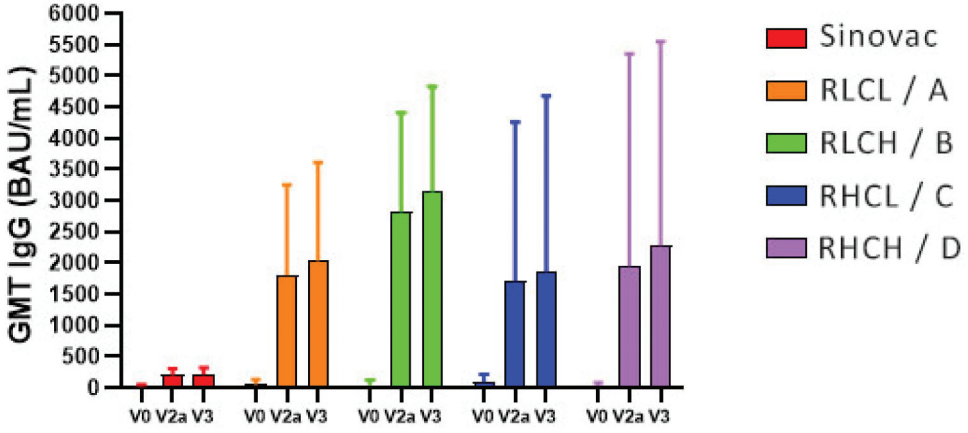


Figure 3. GMT of IgG antibody 14 days and 28 days after the second dose (V0, baseline antibody titer; V2a, 14 days after the second dose vaccination; V3, 28 days after the second dose vaccination).

were not statistically significant ($p = .2341$ for seropositivity, $p = .128$ for GMT) in Figure 5 and Table S3 (Suppl. Data).

By 14 days after the second dose (V2a), all groups demonstrated a marked increase in neutralizing antibody titers. The RLCH group showed the highest GMT (3157.39 IU/mL), closely followed by the RHCH group (2990.80 IU/mL), and the RHCL group (2441.82 IU/mL). In contrast, the Sinovac group remained significantly lower at 299.09 IU/mL. These differences were statistically significant ($p < .0001$). Although seropositive rates were high across all groups (ranging from 86.96% to 100%), the differences were not statistically significant ($p = .6339$). Similarly, while seroconversion rates based on a four-fold increase were highest in the RHCH (86.67%) and RLCH (80%) groups and lowest in the Sinovac group (45.45%), this trend did not reach statistical significance ($p = .1707$) in Figure 5 and Table S3 (Suppl. Data).

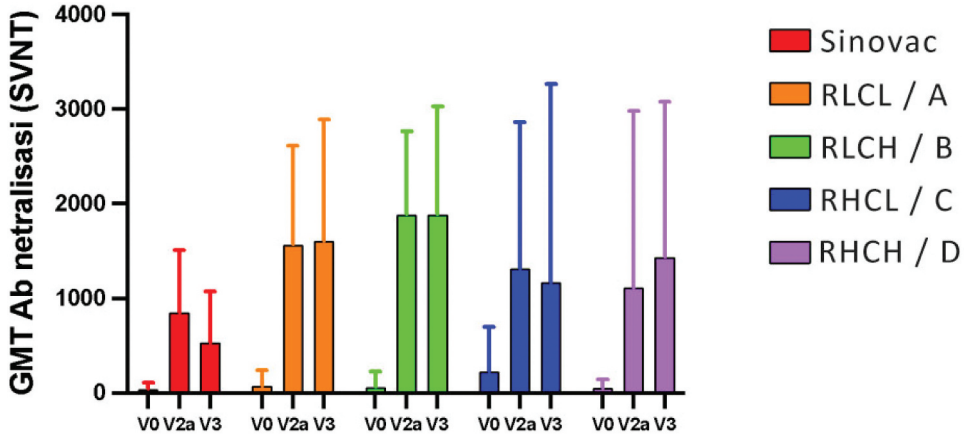


Figure 4. GMT of neutralizing antibody (sVNT) 14 days and 28 days after the second dose. (V0, baseline antibody titer; V2a, 14 days after the second dose vaccination; V3, 28 days after the second dose vaccination).

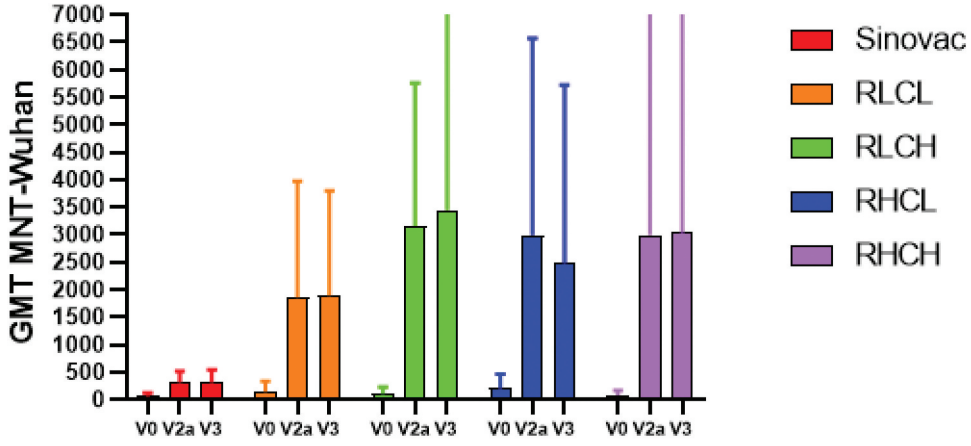


Figure 5. The GMT of neutralization antibody against Wuhan strain 14 days and 28 days after the second dose (V0, baseline antibody titer; V2a, 14 days after the second dose vaccination; V3, 28 days after the second dose vaccination).

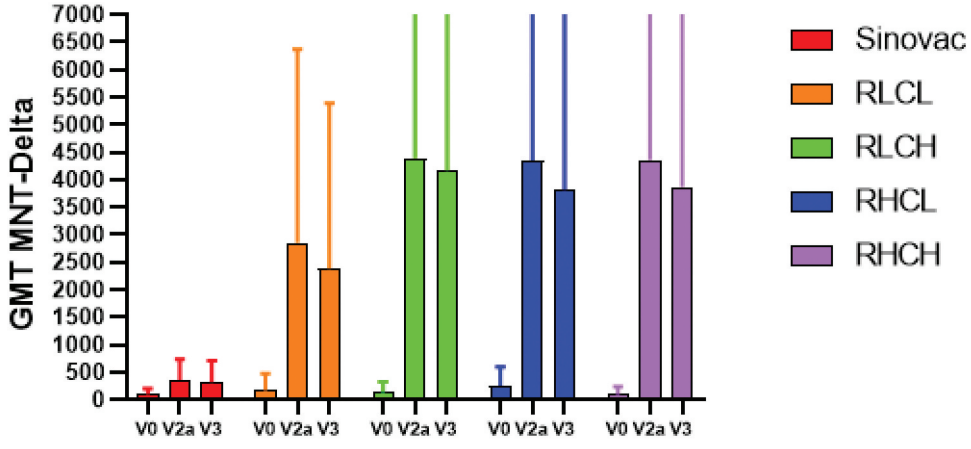


Figure 6. The GMT of neutralization antibody against Delta strain 14 days and 28 days after the second dose (V0, baseline antibody titer; V2a, 14 days after the second dose vaccination; V3, 28 days after the second dose vaccination).

At 28 days post-second dose (V3), neutralizing antibody titers were largely sustained across all groups, with GMTs remaining highest in the RLCH (3442.99 IU/mL) and RHCH (3036.21 IU/mL) groups. The RHCL and RLCL groups showed intermediate responses (2587.02 and 1886.76 IU/mL, respectively), while the Sinovac group again had the lowest GMT (299.10 IU/mL). These differences remained statistically significant ($p < .0001$). Seropositive rates remained high and comparable to V2a levels, with RLCH maintaining 100% seropositivity and the

Sinovac group at 92% ($p = .5214$). Seroconversion rates followed a similar pattern to V2a, with the highest rates observed in the heterologous high-dose groups (RLCH and RHCH, both 80%) and the lowest in the Sinovac group (45.45%), though without statistical significance ($p = .2805$) in Figure 5 and Table S3 (Suppl. Data).

Delta strain. The Figure 6 and Table S4 demonstrate the neutralizing antibody responses against the Delta strain following different vaccination regimens, evaluated at baseline (V0), 14 days (V2a), and 28 days (V3) after the second dose. Prior to vaccination, geometric mean titers (GMTs) were low across all groups, with RHCL showing the highest GMT (256.64 IU/mL) and Sinovac the lowest (89.54 IU/mL). These differences were not statistically significant ($p = .284$). Baseline seropositive rates ranged from 56% (Sinovac) to 79.17% (RHCL), but these differences were also not significant ($p = .422$).

At 14 days post-second dose, neutralizing antibody titers increased substantially in all groups. The highest GMTs were observed in the RLCH (4388.63 IU/mL), RHCL (4350.83 IU/mL), and RHCH (4359.02 IU/mL) groups. The RLCL group followed with a GMT of 2850.20 IU/mL, while the Sinovac group again showed the lowest GMT at 353.24 IU/mL. These differences were highly statistically significant ($p < .0001$). Similarly, median titers reached 7112.29–11784 IU/mL in the heterologous groups, compared to 520.73 IU/mL in the Sinovac group. Although seropositive rates were ≥84% in all groups, including 95.24% for RLCL and 95% for RLCH, these were not statistically different ($p = .6799$). Seroconversion rates, defined as a ≥4-fold increase from baseline, were highest in RHCL (84.21%) and RLCH (80%), and lowest in the Sinovac group (50%), though this difference did not reach statistical significance ($p = .2261$) in Figure 6 and Table S4 (Suppl. Data).

At 28 days post-second dose, antibody titers remained high and stable, with only slight variations compared to V2a. GMTs were again highest in RLCH (4166.23 IU/mL) and RHCH (3864.01 IU/mL), followed by RHCL (3820.58 IU/mL) and RLCL (2377.01 IU/mL), while Sinovac remained the lowest at 311.80 IU/mL. The differences remained statistically significant ($p < .0001$). Median titers at this time point were 5892.00–8332.58 IU/mL in the heterologous groups, versus 520.73 IU/mL in the Sinovac group. The geometric mean ratios (GMRs) comparing day 28 to day 14 were near or slightly above 1.0 in all groups, indicating maintained or slightly increased titers, with the RLCL group showing the highest GMR of 1.20. Seropositive rates remained ≥76% across all groups at V3, and seroconversion rates remained highest in the heterologous groups (≥78%) compared to Sinovac (50%), but these differences again lacked statistical significance (Figure 6 and Table S4).

Discussions

In this study, the subjects were followed up until 6 months after the second dose to assess vaccine safety. Moreover, the subunit vaccine's lack of genetic materials makes them safe and non-infectious/non-viable, making them favorable to be produced. Protein subunit vaccine was the highest against RBD at 87.3% (95% CI 0.671–0.892) 4 weeks after the first dose and 95.6% (95% CI 0.091–0.375) 4 weeks after the second dose.

Any vaccine is expected to cause temporary side effects caused by activation of an immune response and injection site tissue trauma.[17] Co-administration of vaccines with adjuvants is used in virus-like protein (VLP) subunits and certain inactivated vaccines. Adjuvants are essential in inducing specific immune responses, IgG, and Nabs.[18] The non-adjuvanted vaccines display immunopathologic reactions such as high fatigue, vomiting, fever, myalgia, diarrhea, and redness. The alum-adjuvanted CoV vaccine had the lowest systemic side effects among other adjuvants.[19,20] A combination of CpG and Alum adjuvants show myalgia as their side effect with OR 2.42 (95% CI 0.13–44.50). This study showed that the most solicited adverse events (AE) were local pain followed by myalgia,30.40% and 16.00%, respectively. The highest event of local pain was found in group RLCH, followed by group RHCH (RBD dose high, CpG dose high). Groups RLCH and RHCL both had a 20% incidence of myalgia, the highest among the groups. The intensity of the AEs was mostly mild (39.20%). There have not been any serious adverse events related to vaccines up to 6 months of safety follow-up.

This study shows various increments of IgG antibody titers between groups after injection, either the first or second dose, by evaluating blood serum in 175 subjects. Seropositive baselines were found in all five groups (68.00%, 76.19%, 80.00%, 87.50%, and 78.26%). These subjects already had immunity against SARS-CoV-2 before immunization, probably due to asymptomatic infection of COVID-19. An observational study on the anti-SARS-CoV -2 IgG levels reported that the IgG antibody titer can still be detected in 25.6% of asymptomatic cases.[21]

The study found an increase in IgG antibody levels in all groups compared to the baseline after the second injection. Seroconversion from seronegative to seropositive was observed in all groups at 14 and 28 days after the second dose, except group RHCL (RBD dose high, CPG dose low), which does not show full seroconversion (33.33% and 80.00%). All groups showed a 4-fold antibody increase 14 days after the second injection, with the highest increase observed in group RHCH (88.89%). Ultimately, the vaccine candidate is highly tolerable and inducing an immune response for 28 days following complete vaccination. Our recombinant protein subunit vaccine showed similar immunogenicity potency and relatively safe profile with another study.[22] It utilizes the receptor-binding domain (RBD) of SARS-CoV-2 combined with CpG 1018 and aluminum hydroxide as adjuvants – an approach that offers several potential advantages. Subunit vaccines generally have a well-established safety profile, are stable under standard refrigeration, and are suitable for large-scale manufacturing using microbial expression systems (in this case, *Pichia pastoris*), which can be more cost-effective and scalable for use in low- and middle-income countries.[23,24] These attributes are particularly relevant in Indonesia and other resource-limited settings.

Conclusion

In conclusion, based on signs and symptoms, physical examination, and laboratory markers, the new vaccine candidate (Indovac) is well tolerated and has immunogenic potency 28

days after complete vaccination. These findings should be continued to investigate a more significant number of subjects in the potential group of vaccine candidates

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was funded by PT. BioFarma (PERSERO). Funding Number: [002.17/DIR/I/2022 and 004.28/DIR/I/2022].

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Data availability statement

Data availability will be available on the main site of study. Contact the author for future access.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Institutional review board statement

The Health Research Ethics Committee of the Faculty of Medicine Universitas Diponegoro approved the study with number 24/EC/KEPK/FK-UNDIP/I/2022 and also approved by The Ethics Committee of the Faculty of Medicine, University of Indonesia number KET-121/UN2.F1/ETIK/PPM.00.02/2022 and had clinical trial approval from BPOM/NRA with number B.R.G .0106.1.1.02.22.22

References

1. Cawley C, Barsbay MC, Djamangulova T, Erdenebat B, Cilovic-Lagarija S, Fedorchenko V, Gabrani J, Glushkova N, Kalaveshi A, Kandelaki L, et al. The mortality burden related to COVID-19 in 2020 and 2021 - years of life lost and excess mortality in 13 countries and sub-national regions in Southern and Eastern Europe, and Central Asia. Front Public Health. 2024;12:1378229. doi: 10.3389/fpubh.2024.1378229.
2. Yesudhas D, Srivastava A, Gromiha MM. COVID-19 outbreak: history, mechanism, transmission, structural studies and therapeutics. Infection. 2021;49(2):199–213. doi: 10.1007/s15010-020-01516-2.
3. Hedberg P, Parczewski M, Serwin K, Marchetti G, Bai F, Ole Jensen BE, Pereira JPV, Drobniewski F, Reschreiter H, Naumovas D, et al. In-hospital mortality during the wild-type, alpha, delta, and omicron SARS-CoV-2 waves: a multinational cohort study in the EuCARE project. Lancet Reg Health Eur. 2024;38:100855. doi: 10.1016/j.lanepe.2024.100855.
4. Karuniawati A, Pasaribu AP, Lazarus G, Irawany V, Nusantara DU, Sinto R, Nasution MJ, Lubis MR, Nurfitri E, Mutiara M, et al. Characteristics and clinical outcomes of patients with pre-delta, delta and omicron SARS-CoV-2 infection in Indonesia (2020–2023): a multicentre prospective cohort study. Lancet Reg Health Southeast Asia. 2024;22:100348. doi: 10.1016/j.lansea.2023.100348.
5. World Health Organization. Tracking SARS-CoV-2 variants. Indonesia (ID); 2023.
6. Dimonaco NJ, Salavati M, Shih BB. Computational analysis of SARS-CoV-2 and SARS-Like coronavirus diversity in human, bat and pangolin populations. Viruses. 2020 Dec 30;13(1):49.
7. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol. 2022;23(1):3–20. doi: 10.1038/s41580-021-00418-x.
8. Liu X, Xiong J, Sun Z, Hu J, Thilakavathy K, Chen M, Zhao Q, Feng Y, Jiang Q, Xiong C. Omicron: a chimera of two early SARS-CoV-2 lineages. Signal Transduct Targeted Ther. 2022;7(1):90. doi: 10.1038/s41392-022-00949-5.
9. Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin. 2020;41(9):1141–1149. doi: 10.1038/s41401-020-0485-4.
10. Law JLM, Logan M, Joyce MA, Landi A, Hockman D, Crawford K, Johnson J, LaChance G, Saffran HA, Shields J, et al. SARS-COV-2 recombinant receptor-binding-domain (RBD) induces neutralizing antibodies against variant strains of SARS-CoV-2 and SARS-CoV-1. Vaccine. 2021;39(40):5769–5779. doi: 10.1016/j.vaccine.2021.08.081.
11. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, Pan P, Wang W, Hu D, Liu X, et al. Coronavirus infections and immune responses. J Med Virol. 2020;92(4):424–432. doi: 10.1002/jmv.25685.
12. Bayani F, Hashkavaei NS, Arjmand S, Rezaei S, Uskokovic V, Alijanianzadeh M, Uversky VN, Ranaei Siadat SO, Mozaffari-Jovin S, Sefidbakht Y, et al. An overview of the vaccine platforms to combat COVID-19 with a focus on the subunit vaccines. Prog Biophys & Mol Biol. 2023;178:32–49. doi: 10.1016/j.pbiomolbio.2023.02.004.
13. Pusat Krisis Kesehatan Kementerian Kesehatan RI. 70% Penduduk Indonesia ditargetkan menerima vaksin COVID-19 di akhir tahun. Kementrian Kesehatan RI; 2021.
14. Directorate general of disease prevention and control (DG of DPC) of ministry of health of the republic of Indonesia. Annual report COVID-19 2022. 2023.
15. Hu L, Sun J, Wang Y, Tan D, Cao Z, Gao L, Guan Y, Jia X, Mao J. A review of inactivated COVID-19 vaccine development in China: focusing on safety and efficacy in special populations. Vaccines (Basel). 2023;11(6):11. doi: 10.3390/vaccines11061045.
16. Hu Y, Ma C, Wang J. Cytopathic effect assay and plaque assay to evaluate in vitro activity of antiviral compounds against human coronaviruses 229E, OC43, and NL63. Bio Protoc. 2022;12(3):e4314. doi: 10.21769/BioProtoc.4314.
17. Trougakos IP, Terpos E, Alexopoulos H, Politou M, Paraskevis D, Scorilas A, Kastritis E, Andreakos E, Dimopoulos MA. Adverse effects of COVID-19 mRNA vaccines: the spike hypothesis. Trends Mol Med. 2022;28(7):542–554. doi: 10.1016/j.molmed.2022.04.007.
18. Liang Z, Zhu H, Wang X, Jing B, Li Z, Xia X, Sun H, Yang Y, Zhang W, Shi L, et al. Adjuvants for coronavirus vaccines. Front Immunol. 2020;11:589833. doi: 10.3389/fimmu.2020.589833.
19. Pormohammad A, Zarei M, Ghorbani S, Mohammadi M, Razizadeh MH, Turner DL, Turner RJ. Efficacy and safety of COVID-19 vaccines: a systematic review and meta-analysis of randomized clinical trials. Vaccines (Basel). 2021;9. doi: 10.2139/ssrn.3812422.
20. Facciola A, Visalli G, Lagana A, Di Pietro A. An overview of vaccine adjuvants: current evidence and future perspectives. Vaccines (Basel). 2022;10(5):819. doi: 10.3390/vaccines10050819.

21. Yan X, Chen G, Jin Z, Zhang Z, Zhang B, He J, Yin S, Huang J, Fan M, Li Z, et al. Anti-SARS-CoV-2 IgG levels in relation to disease severity of COVID-19. J Med Virol. 2022;94(1):380–383. doi: 10.1002/jmv.27274.
22. Tabarsi P, Anjidani N, Shahpari R, Roshanzamir K, Fallah N, Andre G, Petrovsky N, Barati S. Immunogenicity and safety of SpikoGen®, an adjuvanted recombinant SARS-CoV-2 spike protein vaccine as a homologous and heterologous booster vaccination: a randomized placebo-controlled trial. Immunology. 2022;167(3):340–353. doi: 10.1111/imm.13540.
23. Karbalaei M, Rezaee SA, Farsiani H. Pichia pastoris: a highly successful expression system for optimal synthesis of heterologous proteins. J Cell Physiol. 2020;235(9):5867–5881. doi: 10.1002/jcp.29583.
24. Wang M, Jiang S, Wang Y. Recent advances in the production of recombinant subunit vaccines in Pichia pastoris. Bioengineered. 2016;7(3):155–165. doi: 10.1080/21655979.2016.1191707.