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Research Article

Analysis of Covid-19 Neutralizing Antibody Levels Post Vaccination using Ichroma™ and iFlash Covid-19 Nab

Hartono Kahar[1,*], Sari Prabandari Prasetyaningrum[2], Munawaroh Fitriah[2], Aryati[2], Jusak Nugraha[2]

ABSTRACT

Background: One of the efforts to control SARS-CoV-2 infection in health workers is vaccination. In this study, the levels of SARS-CoV-2 neutralizing antibody (nAb) in health workers were measured with Ichroma and iFlash. **Methods**: This study applied an observational analytic design with a prospective cohort and was conducted at Dr. Soetomo Regional Public Hospital, Surabaya, from January to November 2021. The population of this study included a total of 75 health workers after taking the second dose of the SARS-CoV-2 (Sinovac) vaccine. The Covid-19 NAb levels of the population were tested with Ichroma and iFlash on day 0 before vaccination, as well as days 14 and 28, and months 3 and 6 after vaccination. **Results**: The Friedman test indicated a significant difference in NAb levels according to the iFlash test on day 14, day 28, month 3, and month 6 compared to those before vaccination ($p < 0.05$). The Wilcoxon test revealed a significant difference in NAb levels on day 14, day 28, month 3, and month 6. The results of the Cochran test showed a significant difference in the positivity of NAb according to the Ichroma test on day 14, day 28, month 3, and month 6 compared to those before vaccination ($p < 0.05$). McNemar's test demonstrated that the COI at month 3 was not significantly different from that before vaccination; The COI at month 6 was not significantly different from those at days 14 and 28. The results of the Pearson correlation test and Bland–Altman plot indicated a moderate correlation between Ichroma and iFlash ($r = 0.592$, $p = 0.002$). **Conclusion**: Neutralizing antibodies for Covid-19 were formed after day 14 and started to increase on day 28 and started to decrease in months 3 and 6. The levels of NAb for Covid-19 were measured with Ichroma and iFlash in roughly the same pattern and had a moderate positive correlation.
Key words: Neutralizing Antibody, Ichroma, iFlash.

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INTRODUCTION

The Covid-19 disease caused by the SARS-CoV-2 virus began to develop in Wuhan, continued to spread, and caused a worldwide pandemic.[1,2] A Covid-19 case in Indonesia was first reported on March 2, 2020, and spread throughout Indonesia until July 9, 2020.[3] The Ministry of Health reported 70,736 confirmed cases of COVID-19 with 3,417 deaths (CFR of 4.8%).[4] After the infection of SARS-CoV-2, clinical symptoms begin to appear on days 5-7,[5] and the humoral response in the form of IgM and IgA antibodies can be detected on day five after the initial symptoms appear, and IgG antibodies can be detected after day 14, which start to decrease after month 3.[6]

The antibody titers formed are inversely related to the SARS-CoV-2 viral load, and the levels are higher in patients suffering from critical Covid-19 disease.[5] The disease can be treated in various ways, washing hands, keeping a safe distance, wearing personal protective equipment,[7] vaccinations,[8] and therapy using recombinant interferon (rIFN), tocilizumab, lenzilumab, and a combination of anti-COVID-19 drugs.[6]

One of the efforts to prevent the development of SARS-CoV-2 cases is a vaccine development program.[5] The vaccines developed by Pfizer and Moderna use the basis of mRNA and lipid nanoparticle (LNP) technology, while those developed by AstraZeneca, Johnson, and Gam-Covid-vac (Sputnik) use DNA techniques delivered via recombinant non-replicating adenovirus vectors (AdV). Both types of vaccines encode the production of the SARS-CoV-2 (S) Spike (S) protein, which is the main target for the formation of neutralizing antibody (NAb) and virus-specific T-cell responses as measured in blood 2-4 weeks after inoculation.[9,10]

The vaccines in Indonesia consist of several types of platforms, including (a) inactivated whole virus produced by Sinovac Life Sciences, China,[11] which has high protective efficacy against COVID-19 disease,[12] (b) mRNA-1273 produced by the Moderna with 94.1% efficacy in preventing Covid-19 disease,[13] (c) BNT162b2 produced by BioNTech and Pfizer with 95% efficacy in protecting against Covid-19 disease,[14] and (d) The Oxford-AstraZeneca vaccine that reduces the risk of death by more than 85%, regardless of the variant.[15] Health workers are the spearhead of health services, especially for SARS-CoV-2 patients, and they are susceptible to infection with the disease,[16] thereby they become the first priority for the vaccination program.[17]

Evaluation of the NAb formed due to SARS-CoV-2 is essential to find out which antibodies are formed either naturally or after vaccination to develop protection against Covid-19.[18] Technological developments have introduced many tools to measure antibodies against SARS-CoV-2, including Ichroma and iFlash. iFlash-2019-nCoV Neutralizing Antibody (NAb) uses the chemiluminescent immunoassay method with paramagnetic particles (CLIA) to measure neutralizing antibody levels for the quantitative determination of 2019-nCoV neutralizing antibodies in human serum or plasma

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using the iFlash Immunoassay automatic Analyzer.[19] Ichroma COVID-19 NAb is a fluorescence immunoassay (FIA) to qualitatively detect neutralizing antibodies against Covid-19.[20] This study aims to analyze the levels of NAb against Covid-19 using Ichroma (Boditech Med Inc.) and iFlash Immunoassay Automatic Analyzer (YHLO).

MATERIALS AND METHODS

This study applied an observational analytic design with a prospective cohort and was conducted at the Clinical Pathology Laboratory Unit at the Central Laboratory Installation of Dr. Soetomo Regional Public Hospital, Surabaya, Indonesia, from January to November 2021. Ethical approval for this study was obtained from the Health Research Ethics Committee of Dr. Soetomo Regional Public Hospital, Surabaya, number: 86/113/komitlitkes/V/2021, dated May 21, 2021.

Samples were collected consecutively only from health workers at Dr. Soetomo Regional Public Hospital, Surabaya, who had received two doses of the SARS-CoV-2 (Sinovac) vaccine and never had a history of contracting COVID-19 prior to the vaccination. The participants who agreed to be involved as subjects in this study signed informed consent and were willing to have their blood drawn 5 times on day 0 before vaccination and on day 14, day 28, month 3, and month 6 after the second vaccination (booster). The blood drawn was collected in an SST (serum separator tube). Serum samples collected from the subjects were categorized into the pre-vaccination group (day 0) and the CoronaVac booster post-vaccination group (day 14, 28, month 3, and month 6).

This study was conducted at the Central Laboratory Installation of Dr. Soetomo Regional Public Hospital, Surabaya, from January to October 2021. The population consisted of 75 health workers divided into three groups, namely 25 people for the iFlash test, 25 people for the Ichroma test, and 25 people for the Ichroma equivalence test with iFlash. The population's NAb levels against COVID-19 were tested with Ichroma and iFlash on day 0 before vaccination, as well as days 14 and 28, and months 3 and 6 after vaccination.

The inclusion criteria included male and female health workers at Dr. Soetomo Regional Public Hospital, Surabaya, aged 18-59 years who had received two doses of the SARS-CoV-2 (Sinovac) vaccine, were generally healthy (according to their medical history and physical examination), were willing to follow the entire procedure for six months after the second vaccination dose, were able to understand research procedures, and signed informed consent. The exclusion criteria included participants with a history of SARS-CoV-2 infection and those experiencing SARS-CoV-2 infection during the 6-month study. The samples amounted to 25 people.

The Neutralizing Antibody (NAb) levels generated from iFlash were analyzed using the Friedman test and continued with the Wilcoxon test, while those generated from Ichroma were analyzed using the Cochran test to analyze significant differences in the positivity of NAb levels generated from Ichroma between observation times, followed with the McNemar test. The correlation between the NAb levels generated from Ichroma and iFlash was analyzed using the Pearson correlation test and followed with the Bland-Altman Plot to assess their equivalence presentation.

RESULTS

Participants in this study amounted to 75 people consisting of 29 men and 46 women aged 37.0 ± 8.81 years, respectively. In the Ichroma and iFlash tests, participants aged 34.48 ± 4.341 years are presented in detail in table 1.

The results of the Friedman test showed a significant difference in Nab levels generated from iFlash on day 14, day 28, month 3, and month

Table 1: Characteristics of the samples.

Characteristics of the samples	Ichroma		iFlash		Ichroma vs. iFlash	
Sex	n	%	n	%	n	%
Male	10	40	10	40	9	36
Female	15	60	15	60	16	64
Age (years)						
Mean ± Standard Deviation	37.0 ± 8.81		37.0 ± 8.81		34.48 ± 4.341	

Table 2: Neutralizing Antibody (NAb) levels generated from iFlash.

Observation time	Median (min-max)	P-value
Day 0	0.51 (0–7.76)	
Day 14	47.42 (8.2–313>06)	
Day 28	25.55 (6.89–236.70)	< 0.001
Month 3	8.62 (2.42–935.79)	
Month 6	6.76 (3.74–968.77)	

Table 3: Neutralizing Antibody (NAb) levels generated from Ichroma.

Observation time	Neutralizing Antibody (NAb)		P-value
	Positive	Negative	
Day 0	2 (8%)	23 (92%)	
Day 14	24 (96%)	1 (4%)	
Day 28	18 (72%)	7 (28%)	< 0.001
Month 3	3 (12%)	22 (88%)	
Month 6	20 (80%)	5 (20%)	

Table 4: COI of the Neutralizing Antibody (Nab) levels generated from Ichroma.

Observation time	COI of Neutralizing Antibody (NAb)			P-value
	< 10%	10.0 – 29.9%	≥ 30%	
Day 0	13 (52%)	10 (40%)	2 (8%)	
Day 14		1 (4%)	24 (96%)	
Day 28	3 (12%)	4 (16%)	18 (72%)	< 0.001
Month 3	13 (52%)	9 (36%)	3 (12%)	
Month 6	2 (8%)	3 (12%)	20 (80%)	

Table 5: Ichroma vs iFlash.
Neutralizing Antibody (NAb) levels on month 3 generated from Ichroma and iFlash

Tools	n	Mean ± Standard Deviation
Ichroma (%)	25	59.96 ± 19.827
iFlash (AU/mL)	25	10.27 ± 4.715

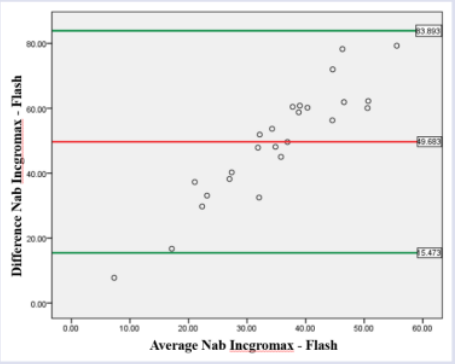

Figure 1: Bland-Altman plot on the NAb levels generated from Ichroma and iFlash.

6 compared to those before vaccination ($p < 0.05$). The results of the follow-up Wilcoxon test indicated that Nab levels on day 14, day 28, month 3, and month 6 were significantly different.

The results of the Cochran test showed a significant difference in the positivity of NAb levels generated from Ichroma on day 14, day 28, month 3, and month 6 compared to that before vaccination ($p < 0.05$). The results of the follow-up McNemar test indicated that the COI on month 3 was not significantly different from that before vaccination; The COI on month 6 was not significantly different from that on days 14 and 28.

The results of the Friedman test revealed a significant difference in the COI of the NAb levels generated from Ichroma between observation times ($p < 0.05$). The results of the follow-up Wilcoxon test indicated that the COI on month 3 was not significantly different from that on day 0; the COI on month 6 was not significantly different from that on days 14 and 28.

The Pearson correlation test resulted in a correlation coefficient of 0.592 with a significance value of 0.002. These results indicate a significant correlation between the COI of the NAb levels generated from Ichroma with the COI of the NAb levels generated from iFlash ($p < 0.05$), with a positive and moderate correlation (0.41–0.60).

The Bland-Altman plot illustrates the correlation between Ichroma and iFlash in measuring NAb levels, revealing that both had moderately correlated results.

DISCUSSION

The health workers in this study were administered with the first and second doses of the Sinovac vaccine. The trial for the second dose of the Sinovac vaccine has been conducted in Turkey, showing efficacy of 91.25%.[21]

In this study, the NAb levels on day 14, day 28, month 3, and month 6t generated from Ichroma and iFlash generally increased compared to those before vaccination. A study conducted by Sadarangani (2021) indicated that after the second dose of vaccine, antibodies on day 28 were detected in 88-97% of participants with a 14-day dosing interval, whereas with an interval of 28 days, antibodies were detected on 99-100% of participants.[22] NAb formed due to covid 19 was detected in 94-100% of individuals on day 28 after the second dose of the vaccine.[22]

Most of the neutralizing antibodies formed due to Covid-19 can bind to the S protein of the virus, preventing it from binding to the host ACE2, thereby preventing infection. First, NAbs will bind to the virus surface protein and then block its interaction with host cell receptors. Second, NAbs bind to viral protein epitopes that interact with host cell coreceptors. Third, NAbs bind to other viral epitopes needed for conformational changes required for membrane fusion.[23] The pattern is similar in both Ichroma and iFlash. A previous study by Mohit et al. (2021) stated that NAb levels could be analyzed using several methods of neutralization assay, including CLIA, Elisa, and LFIA.[24]

The working principle of the two different platform tools for Ichroma to detect antibodies against anti-SARS-CoV-2 is very inconsistent across different platforms of automated chemiluminescent testing. This study demonstrates the diagnostic accuracy of three popular automated platforms and compares their agreement when tested with higher sample sizes. This is the first demonstration of these three platforms and is expected to significantly help the further development of epidemiological strategies to prevent the COVID-19 pandemic as well as in a clinical context.[25]

Antibody production is part of an efficient immune response against SARS-CoV-2, and some antibodies contribute to the effector function of eliminating infectious agents, while others, named NAbs, can neutralize the virus. Most of these antibodies can bind to the viral S protein, preventing it from binding to host ACE2, thereby preventing infection.[23]

Strategies for handling SARS-CoV-2 infection include the administration of appropriate antiviral drugs, vaccination, plasma therapy, and immunomodulatory therapy.[26] The use of Ichroma to monitor six health workers who had a history of Covid-19 infection indicated that on day 14, they showed an increase in NAb levels 2-6 times compared to those before vaccination. On Day 28, four participants showed a decrease in NAb levels, and two participants showed negative NAb levels. On day month 3, these 6 people showed negative NAb levels. On month 6, health workers who were infected with Covid-19 showed an increase in NAb levels up to 99-100%. The main factors that must be considered in selecting laboratory equipment based on the gradation of interest in laboratory services include security, the effectiveness of the learning process, ease of use, accuracy level, and cost.[27]

Ichroma and iFlash have a good security level and are easy to use, but they have different levels of accuracy and cost, which need to be considered by the laboratory in selecting tools according to their respective conditions. iFlash-SARS-CoV-2 is a CLIA paramagnetic particle for detecting IgM and IgG antibodies formed due to the N and S proteins of SARS-CoV-2. iFlash-SARS-CoV-2 was reported to have a sensitivity of less than 50% before day 8 after the onset of the symptoms and increased to 81.8% on days 9-10 after the onset of symptoms for IgM and IgG. A sensitivity of 100% was observed on day 15 or later after the onset of the symptoms when IgG was measured.[24]

The reliability of iFlash in measuring NAb levels formed due to SARS-CoV-2 natural infections and after vaccination reached a sensitivity of 97.9%, a specificity of 94.9%, a positive predictive value of 98.2%, and a negative predictive of 93.8%.[18]

CONCLUSIONS

Neutralizing antibodies for Covid-19 were formed after day 14 and began to increase on day 28, and began to decrease on months 3 and 6. The levels of NAb for Covid-19 were measured with Ichroma and iFlash in roughly the same pattern and had a moderate positive correlation.

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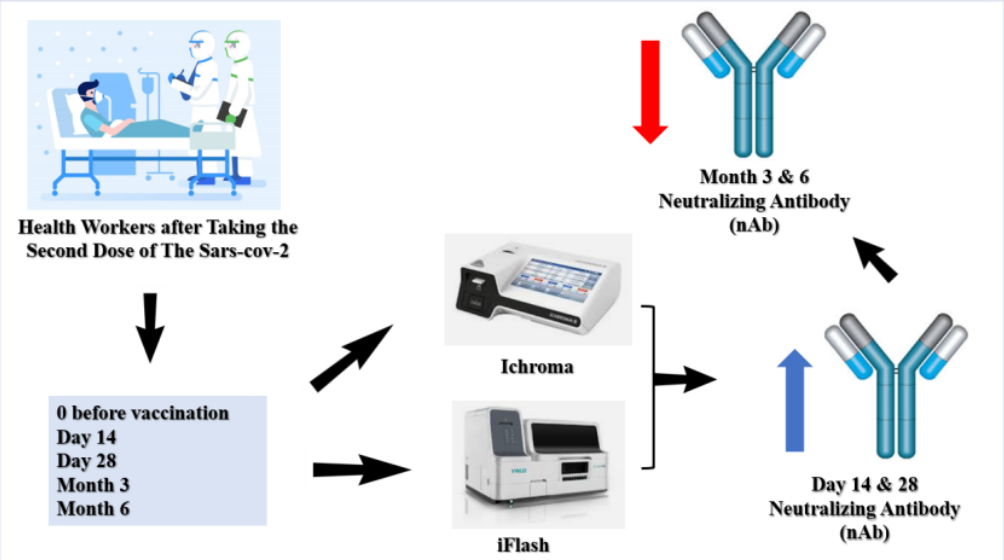
GRAPHICAL ABSTRACT



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