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Research Article

Detection of The Virus and Antibodies of SARS-Cov-2 from Healthy Indonesian Volunteers In 2020: Analysis Using Qualitative and Quantitative Methods

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Abstract

Individuals with COVID-19 and no symptoms, known as asymptomatic carriers, are found mostly in the community. Testing is very important to help reduce the spread of COVID-19. This study aimed to detect virus and antibodies of SARS-CoV-2 in healthy Indonesian volunteers. In September 2020, 45 healthy volunteers participated were taken nasopharyngeal/oropharyngeal swabs to detect the virus SARS-CoV-2 using RT-PCR, and blood samples to detect antibodies qualitatively by three rapid tests (Vazyme, Clugene, and RIGHA kits) and quantitatively by ELISA tests. Among healthy volunteers, COVID-19 asymptomatic carriers who have RT-PCR positive were 16% (7/45) with average of Ct 36.14. The seroprevalence of total SARS-CoV-2 antibodies (IgM and/or IgG) in RT-PCR positivity (asymptomatic carriers) detected by vazyme, clugene and RIGHA kits was 86%, 76% and 52%, respectively and was higher than the negative group (34, 21 and 24%). SARS-CoV-2 IgG antibody titers in the group that were positive for IgM and/or IgG (detected in at least one rapid test) by ELISA was averaged 109.76 ± 114.14 BAU/ml and significantly higher in negative group with titer of 11.45 ± 9.87 BAU/ml. High titer of SARS-CoV-2 IgG anti-RBD antibodies was resulted in consistent positive in three rapid tests (vazyme, clugene, and RIGHA) compared to lower titer. Our findings suggest that healthy individuals with virus and/or antibodies of SARS-CoV-2 may have been infected recently or in the past. Antibody-positive results detected in healthy volunteers by three rapid tests were more common in asymptomatic carriers, and had higher titer antibody.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has occurred globally since the end of 2019 [1]. The disease is caused by the SARS-CoV-2 virus called the novel coronavirus. Over 200 countries have been affected by COVID-19 [2, 3]. The pandemic has also been on for more than a year, and it is not known how long this will be end. COVID-19 spreads easily and most cases are asymptomatic, which has led to a continued increase in cases since it first appeared. Efforts to contain it must detect cases quickly in the community.

Real-time polymerase chain reaction (RT-PCR) is the currently recommended laboratory method for the diagnosis of acute SARS-CoV-2 as the gold standard method [4]. However, the number of RT-PCR machines is limited, and this method requires special equipment and reagents. Additionally, it can also be performed by skilled staff and need several hours. Therefore, the limitations of RT-PCR testing encourage the use of other methods. Instead of using RT-PCR, Indonesia is using some COVID-19 testing methods to improve detection capacity, such as molecular rapid tests (geneXpert methods) and rapid antigen tests. These methods can be used directly to identify the virus that causes COVID-19 infection. However, some alternative methods for mass screening are also widely used in Indonesia, such as rapid antibody and GeNose C19 assays. Screening tests are often simple, fast, and inexpensive to use, but a critical step before they can be used in various settings in certain groups or communities should be test validation, such as for screening healthy people.

COVID-19 antibody tests or known as a serology test is used not for virus detection, but it can determine the presence of antibodies as body's immune response after the infection of virus [5]. The antibodies examined are immunoglobulin M (IgM) as the first line of defense during viral infection and immunoglobulin G (IgG) as adaptive immunity important for long-term immunity and immune memory. In addition, detection of IgM antibodies tends to indicate recent exposure to SARS-CoV-2, while detection of IgG antibodies to COVID-19 indicates exposure to the virus some time ago [5, 6]. Therefore, the detection of antibodies can provide information on natural infections that occurred before the vaccine era, even when there were no symptoms. Moreover, quantitative and qualitative results of antibodies were determined to understand protection against future SARS-CoV-2 infection and the duration of protection [7].

Currently, a large number of antibody tests for COVID-19 are available, and they exhibit different performance capabilities, such as sensitivity, specificity, accuracy, PPV and NPV, depending on the kit or the time of day when the blood sample was collected. Although the usefulness of serological tests is still debated, these tests rapidly help identify asymptomatic carriers of COVID-19 who may spread the virus. This test can also be used to determine the seroprevalence in different populations, assess previous exposure, and contact trace. In this study, we perform a COVID-19 detection in healthy Indonesians using tests for virus using RT-PCR and antibodies using commonly used rapid antibody assays in

Indonesia as qualitative methods (i.e., Vazyme, Clugene and RGHA kits). Additionally, these rapid test results are compared to gold standard RT-PCR to assess validation performance. Moreover, we also quantified the antibodies using an ELISA kit.

Materials and Methods

Sample Collection

We recruited 45 healthy volunteers without any known history of COVID-19 to be included in the study. Nasopharyngeal/oropharyngeal swabs (in 1 mL of viral transport medium) and blood samples (5 mL of peripheral blood) were collected from each individual during September 2020 and then, transported to the Institute of Tropical Diseases, Universitas Airlangga. Specifically, blood samples were left at room temperature for 30 minutes to coagulate, then centrifuged at 1,300 Relative Centrifugal Force (RFC) for 1 minute in a swinging bucket rotor. The serum was then separated and transferred into the clean tubes, and frozen at -80°C until further use. Meanwhile, nasopharyngeal/oropharyngeal swabs were stored directly at -80°C before extraction. Informed consent was obtained from the participants prior to the study. The design of this study was reviewed and approved by the Ethics Committee of Universitas Airlangga Hospital (approval number 163/KEP/2020).

Real Time Polymerase Chain Reaction (RT-PCR) test

Nasopharyngeal/oropharyngeal swabs were utilized to extract the viral RNA of SARS-CoV-2 according to the manufacturer's instructions for the QIAamp Viral RNA Mini Kit (Qiagen; Catalog # 52906, Lot #166024216). The extracted virus RNA was examined by Real-time PCR thermal cycler (Applied Biosytems 7500 fast, software version V2.3 and V2.4) at Institute of Tropical Disease, Universitas Airlangga.

For Real-time PCR, the reaction mixture (20 µL) includes the following reagents: 2 µL of 10x Buffer, 0.25 µL of dNTPs (10 mM each), 0.2 µL of uracil-DNA glycosylase (UDG) (1 U/µL), 0.4 µL of VitaTaq® HS polymerase (2 U/µL), 0.05 µL VitaScript® Enzyme mix including M-MLV (Procomcure, Austria), 0.05 µL of Triton™ X-100 (molecular biology grade, Merck), the primer and probe mixture (for N and S gene of SARS-CoV-2), and RNase/DNase-free ddH_2O up to 20 µL. The mixture was dispensed in 96-well plates (MicroAmp™ Fast Optical 96-well reaction Plate 0.1 mL, Applied Biosystems) and sealed with optical film (MicroAmp™ Optical Adhesive Film, Applied Biosystems). *Pseudoviral* RNAs including viral N and S gene were used as the positive template. Meanwhile, RNase/DNase-free ddH_2O was added to the negative control tubes and used to check any contamination or primer dimer. Then, the Real-time PCR reaction conditions were adjusted as follow: 1) Reverse transcription at 45°C for 5 min, 2) Pre-denaturation at 95°C for 30 sec, 3) 40 cycles of denaturation at 95°C for 5 sec and amplification at 60°C for 30 sec. The reporter dye channel sets as FAM for viral *S* gene and VIC for N gene. The results shows positive when the cycle threshold (Ct) values ≤ 40 for the N and S viral gene regions.

Serological assays to detect SARS-CoV-2 antibodies

Qualitative detection of rapid IgM and IgG antibodies to SARS-CoV-2

Three rapid antibody tests, namely Vazyme (Biotechnology Co., Ltd., China), Clungene® and RI-GHA (Republic of Indonesia - Gadjah Mada-Hepatika-Airlangga, Indonesia) kits, were used in this study and performed according to each of the manufacturer's instructions for the COVID-19 IgM and IgG rapid tests. The results were read after 10 minutes (max 15 minutes), by the naked eye. The test is considered positive if a line is observed on the control and test (IgM and/or IgG) areas. The intensity of the color was not judged.

Quantification detection of anti-SARS-CoV-2 Receptor Binding Domain (RBD) IgG

IgG antibodies were measured with an ELISA method, a two-step chemiluminescence microparticle immunoassays SARS-CoV-2 IgG anti-RBD (SNIBE, Shenzen, China). The assays were performed according to the manufacturer's instructions. Results ≥ 1 AU/mL were considered as positive. The cut-off value in arbitrary units (AU)/mL, the conversion factor to obtain binding antibody unit (BAU)/mL, the cut-off value in BAU/mL and the linearity range in AU/mL are respectively: 1, 4.33, 4.33 and 0.18–100, as declared by the manufacturer. Binding antibody units per milliliter (BAU/mL) proposed by the WHO to standardize any device to the WHO-IS were calculated by applying the conversion factors suggested by the manufacturers, whenever it was possible.

Statistical Analysis

Descriptive statistics were used to describe general information of patients. Continuous data were presented in mean, Standard Deviation (SD), and range. Categorical data were presented in numbers, percentages, and 95% confidence interval (95% CI). Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) were calculated. Two-sided exact P values are reported, and a P value < 0.05 is considered statistically significant. The analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

Forty-five healthy volunteers without any known history of COVID-19 participated in the study and were taken nasopharyngeal/oropharyngeal swabs and blood samples. SARS-CoV-2, the virus that causes COVID-19, was detected in nasopharyngeal/oropharyngeal swabs using RT-PCR. Antibodies against SARS-CoV-2 virus were qualitatively and quantitatively analyzed in blood samples using rapid antibody and ELISA assays, respectively (Figure 1). Our study detected 16% (7/45) of asymptomatic carriers from healthy volunteers who were RT-PCR positive with a mean cycle threshold (CT) value of 36.14 [Table 1].

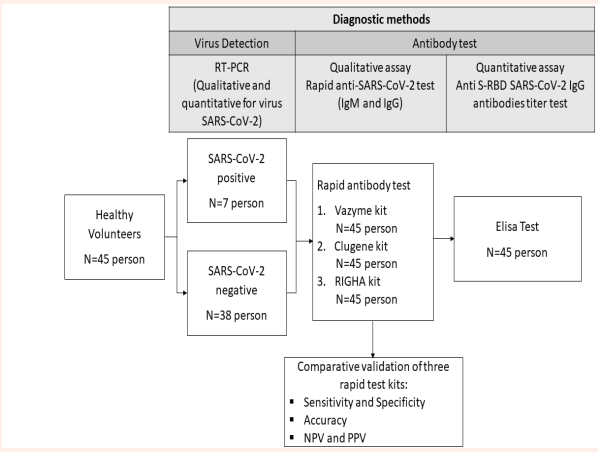

Figure 1: Flowchart of sample collection and testing

The characteristics of healthy volunteers are shown in Table 1 distinguished by RT-PCR results. No different results were found in age, gender, height, weight, and ethics between positive and negative RT-PCR. In general, among RT-PCR positives, antibody-positive results for IgG are more common than for IgM detected by three rapid tests. IgG responses were detected in 86% (6/7) of asymptomatic carriers by vazyme, 71% (5/7) by clugene, and 57% (4/7) by the RIGHA kit. Using vazyme and clugene kits, there was a significant difference in the detection of IgG antibodies in the RT-PCR positive group compared to the negative group. Total antibodies (positive for IgM and/or IgG) were more common in asymptomatic carriers than negative by RT-PCR for the three rapid kits, but only using vazyme and clugene shows significant difference. Analysis of SARS-CoV-2 neutralizing antibodies S-RBD IgG detected by ELISA kits showed that asymptomatic carriers had higher titers than RT-PCR negative groups, but there was no significant difference (Table 1).

Table 1: Baseline Characteristics of Study Participants from Healthy Volunteers based on RT-PCR results

Characteristics	Healthy Volunteers (N=45)		P-value
	RT-PCR +ve (n=7)	RT-PCR -ve (n=38)	
Age (mean ± SD), years	43 ± 15.72	49 ± 19.36	0.440
Sex			
Male	5/7 (71%)	23/38 (61%)	0.462
Female	2/7 (29%)	15/38 (39%)	
Height (mean ± SD), cm	163 ± 10.76	162 ± 8.05	0.757
Weight (mean ± SD), kg	64.33 ± 12.01	60.09 ± 13.47	0.476
Etnics			
Javanese	6/7 (86%)	29/38 (76%)	0.341
Madura	1/7 (14%)	1/38 (3%)	
Chinese	0/7 (0%)	1/38 (3%)	
Unknown	0/7 (0%)	7/38 (18%)	
Ct value (by RT-PCR detection)	36.14 ± 1.91	-	-
Rapid test			
Vazyme			
IgM reactive	1/7 (14%)	3/38 (8%)	0.505
IgG reactive	6/7 (86%)	11/38 (29%)	0.008*
IgM and/or IgG reactive	6/7 (86%)	13/38 (34%)	0.017*
IgM and IgG non-reactive	1/7 (14%)	25/38 (66%)	0.017*
Clugene			
IgM reactive	3/7 (43%)	6/38 (16%)	0.131
IgG reactive	5/7 (71%)	8/38 (21%)	0.015*
IgM and/or IgG reactive	5/7 (71%)	8/38 (21%)	0.015*
IgM and IgG non-reactive	2/7 (29%)	30/38 (79%)	0.015*
RGHA			
IgM reactive	2/7 (29%)	5/38 (13%)	0.296
IgG reactive	4/7 (57%)	9/38 (24%)	0.093
IgM and/or IgG reactive	4/7 (57%)	9/38 (24%)	0.093
IgM and IgG non-reactive	3/7 (43%)	29/38 (76%)	0.093
Neutralizing antibodies, SARS-CoV-2 S-RBD IgG detected by ELISA test (BAU/mL)[a]	85.09 ± 98.38	47.04 ± 86.43	0.300

aElisa test for antibody titer, BAU/ml as WHO standard unit
*p-value < 0.050 is defined as significant results

(Table 2) presents a comparison of qualitative methods for antibody detection using three rapid tests. In healthy volunteers, 9%, 20%, and 16% of IgM antibodies were detected using the vazyme, clugene, and RIGHA kits, respectively, while IgG antibodies were detected in 38%, 29%, and 29%, respectively. Total antibodies (IgM and/or IgG) were identified in 43%, 29% and 29% by vazyme, clugene and RIGHA kits, respectively. Due to differences in IgM, IgG or IgM and/or IgG assay results, performance validation of the three rapid assays needs to be evaluated. The highest sensitivity test for IgM detection was 43% (95% CI, 9.9-81.6%) using clugene kit and for IgG as well as IgM and/or IgG detection was 86% (95% CI, 42.1-99.6%) using vazyme kit. The highest specificity test for IgM detection was 92% (95% CI, 78.6-98.3%) using vazyme and for IgG as well as IgM and/or IgG was 79% (95% CI, 62.7-90.4%) using clugene. All three rapid tests showed Negative Predictive Value (NPV) percentages above 90%, but the Positive Predictive Value (PPV) was less than 50%. However, three tests were found to have accuracy values of 69% (95% CI, 0.598-0.917%), 78% (95% CI, 0.560-0.944%) and 73% (95% CI, 0.458-0.877%), respectively.

Table 2: Analysis qualitative methods for antibody detection using rapid tests

	Rapid antibody tests		
	Vazyme kit (N=45)	Clugene kit (N=45)	RGHA kit (N=45)
Frequency, n (%)			
IgM reactive, n/N (%) (95% CI)	4/45 (9%) (2.2 – 17.8%)	9/45 (20%) (8.9 – 33.3%)	7/45 (16%) (6.7 – 26.7%)
IgG reactive, n/N (%) (95% CI)	17/45 (38%) (24.4 – 51.1%)	13/45 (29%) (15.6 – 42.2%)	13/45 (29%) (17.8 – 42.2%)
IgM and/or IgG reactive, n/N (%) (95% CI)	19/45 (42%) (28.9 – 55.6%)	13/45 (29%) (15.6 – 42.2%)	13/45 (29%) (17.8 – 42.2%)
IgM and/or IgG non-reactive, n/N (%) (95% CI)	26/45 (58%) (44.4 – 71.1%)	32/45 (71%) (57.8 – 84.4%)	32/45 (71%) (57.8 – 82.2%)
Sensitivity, % (95% CI)			
IgM reactive	14% (0.361 – 57.9%)	43% (9.9 – 81.6%)	29% (3.67 – 71%)
IgG reactive	86% (42.1 – 99.6%)	71% (29 – 96.3%)	57% (18.4 – 90.1%)
IgM and/or IgG reactive	86% (42.1 – 99.6%)	71% (29 -96.3%)	57% (18.4 – 90.1%)
Specificity, % (95% CI)			
IgM reactive	92% (78.6 – 98.3%)	84% (68.7 – 94%)	87% (71.9 – 95.6%)
IgG reactive	71% (54.1 – 84.6%)	79% (62.7 – 90.4%)	76% (59.8 – 88.6%)
IgM and/or IgG reactive	66% (48.6 – 80.4%)	79% (62.7 – 90.4%)	76% (59.8 – 88.6%)
Positive Predictive Value, % (95% CI)	32% (12.6 – 56.6%)	38% (13.9 – 68.4%)	31% (9.09 – 61.4%)
Negative Predictive Value, % (95% CI)	96% (80.4 – 99.9%)	94% (79.2 – 99.2%)	91% (75 – 98%)
Accuracy, % (95% CI)	69% (0.59 – 0.91%)	78% (0.56– 0.94%)	73% (0.45 – 0.87%)

Our study also showed SARS-CoV-2 neutralizing antibody S-RBD IgG titers compared with the two groups divided based on rapid antibody test results. When healthy volunteers were grouped according to antibody rapid test results, groups with IgM and/or IgG reactivity results (detected in at least one rapid test) had higher neutralizing titers than non-reactivity. In addition, there were also significant differences between these groups (p-value 0.000) (Table 3).

Table 3: Analysis quantitative methods for antibody detection using Elisa kit

	IgM and/or IgG reactive by rapid tests (at least detected in one rapid test) (n=19)	IgM and/or IgG non-reactive by rapid tests (n=26)	p-value
Neutralizing antibodies titer, SARS-CoV-2 S-RBD IgG detected by ELISA test (BAU/mL)[a]	109.76 ± 114.14	11.45 ± 9.87	0.000*

aElisa test for antibody titer, BAU/mL as WHO standard unit
*p-value < 0.050 is defined as significant results

Antibody test results for asymptomatic carriers were compared between qualitative and quantitative methods, as described in (Table 4). Our results showed that IgG antibodies were consistently detected across the three rapid assays when the ELISA assay detected higher titers of anti-SARS-Cov-2 S-RBD IgG, seen in sample HV1-4 with antibodies titers range 34.64 - 251.57 BAU/mL, and in sample HV8-19 with antibodies titers range 57.60 - 332 BAU/mL in (Table 4). One sample (HV7) was positive by PCR, but all three rapid antibody tests were negative, and the ELISA test titer was the lowest (8.23 BAU/mL).

Table 4: Profile of qualitative and quantative analysis of virus and/or antibodies of SARS-CoV-2

RT-PCR detection	Samples	Gender	Age	Vazyme kit		Clugene kit		RGHA kit		ELISA kit (titer, BAU/mL)	PCR (Ct value)
				IgM	IgG	IgM	IgG	IgM	IgG		
RT-PCR Positive	HV1	Male	30	-	R	-	R	R	R	251.57	37.44
	HV2	Male	66	-	R	R	R	R	R	192.25	37.35
	HV3	Female	58	R	R	R	R	-	R	82.27	32.89
	HV4	Female	53	-	R	-	R	-	R	34.64	35.89
	HV5	Male	32	-	R	R	R	-	-	12.99	37.13
	HV6	Male	36	-	R	-	-	-	-	10.39	33.50
	HV7	Male	26	-	-	-	-	-	-	8.23	35
RT-PCR Negative	HV8	Female	44	R	R	R	R	R	R	332	-
	HV9	Female	52	-	R	R	R	-	R	264	-
	HV10	Female	8	-	R	-	R	R	R	259	-
	HV11	Female	32	-	R	R	R	R	R	240	-
	HV12	Female	33	-	R	R	R	R	R	207	-
	HV13	Male	54	-	R	R	R	-	R	57.60	-
	HV14	Female	41	-	R	R	R	-	-	33.30	-
	HV15	Female	22	-	R	-	R	-	-	27.71	-
	HV16	Male	66	-	R	-	-	-	-	23.82	-
	HV17	Female	30	-	R	-	-	-	R	14.72	-
	HV18	Female	56	R	-	-	-	R	R	6,93	-
	HV19	Male	60	-	R	-	-	-	-	1,73	-

HV: Healthy Volunteer
R: Reactive detected by rapid tests

Discussion

Indonesia detected its first case of COVID-19 since March 2020. Furthermore, COVID-19 is spreading rapidly, especially in Java, eventually spreading across the region [8, 9]. In the beginning, the number of tests is limited due to lack of detection tools and so many cases arise[10]. As a result, the government has issued policies related to some of the COVID-19 testing methods used in Indonesia, such as real-time PCR, molecular rapid tests (geneXpert method), rapid antibody and antigen tests, and Genose C19. These methods are used to increase reliable testing capabilities. However, the validity and accuracy of the examination method must be ensured. In order to study it, the results of some of the methods used to detect COVID-19 must be compared to the gold standard real-time PCR [11].

During pandemic COVID-19, case finding is very important, so testing is critical to breaking chains of transmission [12]. In the middle of 2020, Indonesian government also allows to use a rapid antibody test for screening COVID-19 suspected people, because the gap between the real-time PCR testing capacity in laboratories and the number of suspected cases to be tested continues to be a major issue. The rapid antibody test is not as sensitive as real-time PCR, but it is easy to use and has a faster turnaround time, with results in 30 minutes. It can also be performed directly at the point of care, so a Biosafety Level 2 (BSL2) laboratory facility is not required. The method principle of rapid antibody tests differs from diagnostic tests such as real-time PCR, molecular rapid tests (geneXpert methods) and rapid antigen tests that directly detect the virus. Antibody tests, also known as serology tests, determine whether you have antibodies as the body's response against the virus infects you now or in the past. [13] Antibody tests cannot be used to diagnose COVID-19, but only for screening. Because, a test to diagnose COVID-19 determines if you only currently have the disease. Many antibody tests are recently in development or available for use to detect anti-SARS-CoV-2. The timing of antibody test also affects accuracy. When testing too early in the course of infection and the immune response is still building up in your body, the test may not detect antibodies. Then, antibody testing is not recommended until at least 2 to 3 weeks after your symptoms started. [13] Most of these studies show that people who have recovered from infection have antibodies to the virus. [14, 15] With antibody testing, it is possible to test negative but actually be infected (false negative result) or test positive but not be infected (false positive result). Hence, to understand about the possibility of COVID-19 infection in healthy people now or in the past, our study was to detect SARS-CoV-2 virus and qualitatively and quantitatively detecting SARS-CoV-2 antibodies and then observe how these assays performed. The three commercial rapid antibody tests used were Vazyme, Clugene, and RIGHA kits for qualitative methods, followed by validation performance and ELISA assays for quantitative methods.

In the present study, asymptomatic carriers who tested positive for COVID-19 were found in 16% (7/45) of healthy individuals. Asymptomatic carriers indicate that the SARS-CoV-2 virus is present and possibly multiplying, and although there is no

clinical response, this recent infection may be contagious [16]. The percentage of asymptomatic infections in the population tested may vary between studies conducted in different locations and communities. The high percentage of asymptomatic infections highlights the potential transmission risk of asymptomatic infections in communities [17]. Many studies showed that high prevalence of COVID-19 cases is asymptomatic. Asymptomatic person seem to account for about 40 to 45 percent of SARS-CoV-2 infections, and they can spread the virus to others for long periods of time, possibly more than 14 days [18]. In a meta-analysis of 16 studies including 2,788 patients with SARS-CoV-2 infection, the prevalence of asymptomatic cases was 48.2% (95% CI, 30-67%) [16]. Most of the asymptomatic carriers (470 of 624, 75.3%) were 'close contacts' of symptomatic subjects (428 of 470, 91%) [19]. The limitations of diagnostic tools lead us to consider that testing should be done in emergency communities, such as 'close contacts' with people infected with SARS-CoV-2 or person with any symptoms of COVID-19. However, to understand the spread of COVID-19, we need to examine the entire population. Our findings suggest that asymptomatic cases (16%) have been identified in healthy people, although not as many as have been reported in close contact with people infected with SARS-CoV-2.

Overall, the seroprevalence of total SARS-CoV-2 antibodies (IgM and/or IgG) in healthy volunteers was 42% (19/45) using the vazyme kit showing the highest prevalence. Antibody (IgM and/or IgG) detection rates in the asymptomatic group (RT-PCR positive), were 86%, 76%, and 52% with vazyme, clugene, and RIGHA kits, respectively, and higher than in the negative group (34%, 21%, and 24%). Healthy individuals with antibodies to SARS-CoV-2 indicated that they were currently infected with the SARS-CoV-2 virus and no symptoms (asymptomatic case), as evidenced by the virus detected, while they who did not have the virus may have been infected in the past and did not realize. High antibody positivity rates found in healthy people may also indicate high infection rates in the population, including asymptomatic people and previous infection. The findings from previous studies have shown that in East Java, Indonesia, the COVID-19 outbreak among asymptomatic people is characterized by a high infection rate [20]. Antibodies IgG only as well as IgM and/or IgM reactivity were frequently detected in asymptomatic carriers by all rapid kits, and was significantly detected by vazyme and clugene kits compared to RT-PCR negative group. This result is similar to the study which found that asymptomatic patients had significantly higher rates of IgM and IgG positivity than healthy controls [21].

The study also compared the results of the SARS-CoV-2 rapid antibody test and the RT-PCR test using samples from healthy volunteers to examine the validation performance of the three rapid tests used, such as the sensitivity, specificity, PPV, NPV, and accuracy. According to the manufacturer's data, the sensitivities for the rapid detection of IgM and IgG were 66.7% and 96.7%, 96.7% and 100%, and 96.8% and 74% for vazyme, clugene and RIGHA kits, respectively. While, the specificity of IgM and IgG are 77.5% and 90%, 98% and 100%, 96.8% and 74% for vazyme, clugene and RIGHA kits, respectively. Our results, the sensitivity of IgM and IgG detection using vazyme, clugene and RIGHA kits were obtained 14% and 86%, 43% and 71%, and 29% and 76%, respectively. While, the specificity of IgM and IgG detection using vazyme, clugene, and RIGHA kits were obtained 92% and 71%, 84% and 79%, and 87% and 76%, respectively. The highest sensitivity of using vazyme was used for IgG detection and the highest specificity of using clugene for IgM detection. The results suggest that these sensitivities and specificities of rapid tests may be affected by the criteria of sample use, the number of samples, and the location where the samples were collected. The sensitivity of the three rapid tests performed using our samples was below company standards [13]. It is because that sensitivity has mainly been evaluated in hospitalised patients, so it is unclear whether the tests are able to detect lower antibody levels likely seen with milder and asymptomatic COVID-19 disease [13]. The sensitivity of antibody tests is too low in the first week since symptom onset to have a primary role for the diagnosis of COVID-19, but they may still have a role complementing other testing in individuals presenting later, when RT-PCR tests are negative, or are not done [13]. Three rapid tests had higher NPV (>90%) than PPV (<50%), suggesting that these kits may be useful in detecting true negatives, rather than true positives, for COVID-19 infection in healthy volunteers. Therefore, rapid tests are suitable for screening rather than diagnostic tools. However, serological assays can be used to support nucleic acid amplification tests [22].

Quantitative method for IgG antibody detection was measured with an ELISA method, a two-step chemiluminescence microparticle immunoassays SARS-CoV-2 IgG anti-RBD. RBD is a promising target for detecting anti-SARS-CoV-2 antibodies as it has a highly distinct sequence compared to seasonal coronaviruses and induces robust antibody response. RBD based IgG assays show high concordance with virus neutralization assays [23]. Our results show that on average the SARS-CoV-2 IgG anti-RBD titers detected by ELISA were significantly higher in the IgM and/or IgG positive group (109.76 ± 114.14 BAU/mL) detected by at least one of the rapid tests than in the negative group (11.45 ± 9.87 BAU/mL). The antibody titers may determine whether the rapid antibody results are positive or not. SARS-CoV-2 IgG anti-RBD antibodies with high titer resulted in consistent positive in three rapid tests (vazyme, clugene, and RIGHA) compare to lower titer, studies are needed to determine cutoff values of antibodies titer for each rapid testing. Antibodies detection assays are useful for sero-surveillance studies, such as the individual risk assessment, evaluation of the sustainability of antibodies after infection or vaccination, and for determining the need for booster dose in the post-vaccine era. [23, 24] At the population level, the serosurveillance studies to understand the extent of COVID-19 transmission in the community.

Conclusion

Our findings indicate that virus and antibodies of SARS-CoV-2 have been identified in healthy volunteers, showing people suspected of current or previous SARS-CoV-2 infection. Antibody-positive results detected by three rapid tests were more common in asymptomatic carriers, and had higher titer antibody.

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