Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection and Chemotherapy 28 (2022) 266–272

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: www.elsevier.com/locate/jic

Original Article

Interpretations of SARS-CoV-2 IgM and IgG antibody titers in the seroepidemiological study of asymptomatic healthy volunteers☆

Akihisa Mitani [a,*,1], Takeshi Horie [a,1], Rin Yokoyama [b], Yuki Nakano [b], Kensuke Hamada [a], Yukiko Inoue [a], Minako Saito [a], Takashi Ishii [a], Mitsuhiro Sunohara [a], Ryota Takahashi [a], Tomoko Usui [a], Noriko Emoto [a], Nahoko Nishimoto [a], Yoko Murano [a], Sachiko Okazaki [a], Shoko Tateishi [a], Kuniaki Iwasawa [a], Atsushi Yao [a], Makoto Kurano [b], Yutaka Yatomi [b], Shintaro Yanagimoto [a]

[a] Division for Health Service Promotion, the University of Tokyo, Japan
[b] Department of Clinical Laboratory, the University of Tokyo Hospital, Japan

ARTICLE INFO

Keywords:
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
Seroepidemiological study
Asymptomatic volunteers
iFlash-SARS-CoV-2 IgM and IgG
Elecsys anti-SARS-CoV-2 Ig
Architect SARS-CoV-2 IgG

ABSTRACT

Introduction: The usefulness of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody tests in asymptomatic individuals has not been well validated, although they have satisfied sensitivity and specificity in symptomatic patients. In this study, we investigated the significance of IgM and IgG antibody titers against SARS-CoV-2 in the serum of asymptomatic healthy subjects.

Methods: From June 2020, we recruited 10,039 participants to the project named the University of Tokyo COVID-19 Antibody Titer Survey (UT-CATS), and measured iFlash-SARS-CoV-2 IgM and IgG (YHLO IgM and IgG) titers in the collected serum. For the samples with increased IgM or IgG titers, we performed additional measurements using Elecsys Anti-SARS-CoV-2 Ig (Roche total Ig) and Architect SARS-CoV-2 IgG (Abbott IgG) and investigated the reactivity to N, S1, and receptor binding domain (RBD) proteins.

Results: After setting the cutoff value at 5 AU/mL, 61 (0.61%) were positive for YHLO IgM and 104 (1.04%) for YHLO IgG. Few samples with elevated YHLO IgM showed reactivity to S1 or RBD proteins, and IgG titers did not increase during the follow-up in any samples. The samples with elevated YHLO IgG consisted of two groups: one reacted to S1 or RBD proteins and the other did not, which was reflected in the results of Roche total Ig.

Conclusions: In SARS-CoV-2 seroepidemiological studies of asymptomatic participants, sufficient attention should be given to the interpretation of the results of YHLO IgM and IgG, and the combined use of YHLO IgG and Roche total Ig might be more reliable.

1. Introduction

A cluster of undiagnosed pneumonia, which was subsequently called the coronavirus disease 2019 (COVID-19), was first reported in December 2019 in China [1]. The disease spread across the world very quickly, and even in Japan, since the first case was reported in January 2020, the number of infected people is still increasing. After the relatively small two infection waves, the third wave of infection had begun began in early November. As of March 2021 in Japan, a total of 450,000 cases were confirmed, with a total death toll exceeding 8700 [2].

There are three main types of diagnostic methods for this disease: viral gene detection by reverse transcription polymerase chain reaction (RT-PCR), viral antigen detection, and human antibody detection. It is recommended that RT-PCR or antigen test be performed to get a definitive diagnosis in symptomatic individuals [3,4]. Conversely, due to the window period until the development of serum antibodies, antibody testing is not suitable for the diagnosis of the acute phase [5,6]. On the other hand, it has been reported that asymptomatic individuals seem to account for approximately 40%–45% of SARS-CoV-2 infections, and they can possibly infect others over a long period of time [7]. Hence,

☆ All authors meet the ICMJE authorship criteria.

* Corresponding author. Hongo 7-3-1, Bunkyo-ku, Tokyo, 113-0033, Japan.
E-mail address: mitania-tky@umin.ac.jp (A. Mitani).

[1] contributed equally.

https://doi.org/10.1016/j.jiac.2021.11.020
Received 9 September 2021; Received in revised form 2 November 2021; Accepted 22 November 2021
Available online 7 December 2021

epidemiological monitoring of only symptomatic individuals can only control a portion of all infections. By examining the antibody prevalence in each region and community, it is possible to know the status and trends of infection up to that point. ~~Antibody testing, however, has not yet been established as a screening modality for early infection, although it is considered to be useful for widespread screening for epidemiological surveys in asymptomatic individuals.~~

The SARS-CoV-2 has four structural proteins: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins [8]. The S and N proteins show high antigenicity. The S protein is the main target of neutralizing antibodies. The S protein is functionally divided into two subunits (S1 and S2). The S1 domain comprises the receptor binding domain (RBD), which is responsible for binding to the angiotensin-converting enzyme 2 membrane receptor of the host cell. The N protein is a structural component of the helical nucleocapsid and related to viral pathogenesis, replication, and RNA packaging [8].

A variety of types of test are currently available for the detection of antibodies, including IgM, IgG, and IgA against the S protein, N protein, and RBD. Most tests are based on IgM and IgG, and there are still few studies based on IgA [9]. Many of these kits generally have good sensitivity and specificity in symptomatic patients [5,10–13]. However, the reliability and validity of the asymptomatic population have not been sufficiently confirmed; accuracy problems and mutual comparison between kits are challenges in this population subset. In general, it is known that sensitivity and specificity decrease in populations with low prevalence. Therefore, it is common to evaluate a combination of multiple test agents. For example, a survey in Tokyo defined positives as only those samples that tested positive for two different testing kits [14,15].

We measured IgG and IgM titers against SARS-CoV-2 in the serum of members of the University of Tokyo using the SARS-CoV-2 IgG and IgM reagents (iFLASH) (Shenzhen YHLO Biotech, Shenzhen, China), in the project named the University of Tokyo COVID-19 Antibody Titer Survey (UT-CATS), which main purpose is estimating seroprevalence in our university. We previously reported that the serum levels of these antibodies are affected by several factors, including age, smoking habits, and comorbidities [16].

The present study aimed to investigate how to interpret the results of SARS-CoV-2 IgM and IgG assays as a screening test for asymptomatic individuals by additional measurements, including antibody titers measured by other methods and the reactivity to N, S1, and RBD proteins of SARS-CoV-2.

2. Method

2.1. Subjects and sample collection

We recruited 10,039 asymptomatic subjects among students, staff, and faculty members of the University of Tokyo, Japan between June 11 and December 22, 2020, as part of the project UT-CATS. All participants were 18 years or older. The aims of this study were explained, and all participants provided written informed consent. Five milliliters of blood samples were collected at health service centers or health checkup venues in the University of Tokyo, or the University of Tokyo Hospital. The participants were asked to answer an online questionnaire on whether or not they had a past history of COVID-19.

The Clinical Research Review Board of the University of Tokyo approved the study protocol (Registration number: 2020052NI). All methods were carried out in accordance with relevant guidelines and regulations.

2.2. Measurement kits

iFlash-SARS-CoV-2 IgM and IgG (YHLO Biotechnology Company, Ltd., Shenzhen, China).

The serum titers of SARS-CoV-2 IgM and IgG of all subjects were measured using the iFlash 3000 fully automatic chemiluminescence immunoassay analyzer (YHLO Biotechnology Company, Ltd., Shenzhen, China). We used the SARS-CoV-2 IgM and IgG kits containing magnetic beads coated with SARS-CoV-2 N protein and S protein. According to the manufacturer's instructions, we set the cutoff of IgM and IgG at 10.0 AU/mL [10]. Data below detection limit (0.20 AU/mL) were considered as 0.20 in the subsequent analyses. Previous studies suggested that the actual cutoff could be lower than 10.0 AU/mL [10]. Therefore, in this study, we performed the additional tests cited below on all samples with either IgM or IgG titer of 5.0 AU/mL or higher.

Table 1
Distributions of YHLO IgM and IgG titers.

YHLO IgM (AU/mL)		
0.00–0.99	9154	91.18%
1.00–1.99	645	6.42%
2.00–4.99	179	1.78%
5.00–9.99	38	0.38%
10.00-	23	0.23%
Total	10,039	100.00%
YHLO IgG (AU/mL)		
0.00–0.99	8666	86.32%
1.00–1.99	941	9.37%
2.00–4.99	328	3.27%
5.00–9.99	57	0.57%
10.00-	47	0.47%
Total	10,039	100.00%

To measure the reactivity to N, S1, and RBD proteins, we used magnetic beads coated with each single antigen (YHLO Biotechnology Company, Ltd., Shenzhen, China). Measuring method is the same as the SARS-CoV-2 IgM and IgG kits, but this shows Ig titers against N, S1, and RBD proteins individually. Cutoff values have not yet been set.

The remaining serum was stored at −80 °C. In order to confirm the reproducibility of the results, IgM and IgG were measured again in some samples one to three months after the first measurement.

2.3. Elecsys Anti-SARS-CoV-2 (Roche diagnostics International AG, Rotkreuz, Switzerland)

The electrochemiluminescence immunoassay was used for identifying total antibodies against SARS-CoV-2 N protein while not differentiating between IgA, IgM, or IgG antibodies. The manufacturer set the cutoff index (COI) at 1.0.

2.4. Architect SARS-CoV-2 IgG (Abbott Laboratories, Illinois, United States)

The chemiluminescent microparticle immunoassay (CMIA) is intended for the qualitative detection of IgG against N protein of SARS-CoV-2. The manufacturer set the cutoff at 1.4 index (S/C).

2.5. Statistical analyses

Antibody titers between subgroups were compared using the Mann–Whitney test or the Wilcoxon matched-pairs signed ranks test. The Spearman's rank test was used for examining the degree to which two data sets of antibody titers were correlated.

We performed all statistical analyses using Prism version 7.00 (GraphPad). The results are expressed as the mean ± standard error of the mean unless otherwise stated. All tests were two-tailed, and a p value < 0.05 was considered to be statistically significant.

Table 2
The measurements of Roche total Ig and Abbott IgG titers.

		total	Roche total Ig		Abbott IgG	
			≧1.0 COI		≧1.4 index (S/C)	
YHLO IgM (AU/mL)	5.00–9.99	38	[a]1	2.63%	[a]1	2.63%
	10.00-	23	0	0.00%	[a]1	4.35%
	total	61	[a]1	1.64%	[a]2	3.28%
YHLO IgG (AU/mL)	5.00–9.99	57	4	7.02%	4	7.02%
	10.00-	47	29	61.70%	25	53.19%
	total	104	33	31.73%	29	27.88%
both negative (9,876)		1204	1	0.08%	1	0.08%

[a] also increased YHLO IgG.

3. Results

3.1. Distributions of YHLO IgM and IgG titers

We included 10,039 asymptomatic volunteers in this study. There were 5111 (50.9%) males and 4928 (49.1%) females. Average age (±standard deviation) was 38.2 ± 12.8 years old.

We measured the serum titers of iFlash-SARS-CoV-2 IgM and IgG (YHLO IgM and IgG), and distributions of the titers are summarized in Table 1. Most of the samples showed IgM and IgG titers below 2.0 AU/mL. According to the manual provided by the manufacturer, both cutoff values for IgG and IgM titers are 10.0 AU/mL. Twenty-three participants (0.23%) showed IgM titers above this cutoff, whereas the IgG titers of 47 participants (0.47%) were over the cutoff.

It has been reported that the cutoff values might be lower than the manufacturer's reported cutoff in Japanese patients with symptomatic COVID-19 [10]. Using the cutoff value of 5.0 AU/mL, the number of positive participants was 61 (0.61%) for IgM and 104 (1.04%) for IgG.

Only two samples showed titers of more than 5.0 AU/mL titers for both IgM and IgG, and there was no correlation found between IgM and IgG titers (r = 0.01).

3.2. Roche total Ig titers and Abbott IgG titers

In 163 samples, which showed 5.0 AU/mL or more titers of YHLO IgM or YHLO IgG, we measured Elecsys Anti-SARS-CoV-2 Ig (Roche total Ig) and Architect SARS-CoV-2 IgG (Abbott IgG) (Table 2). We considered

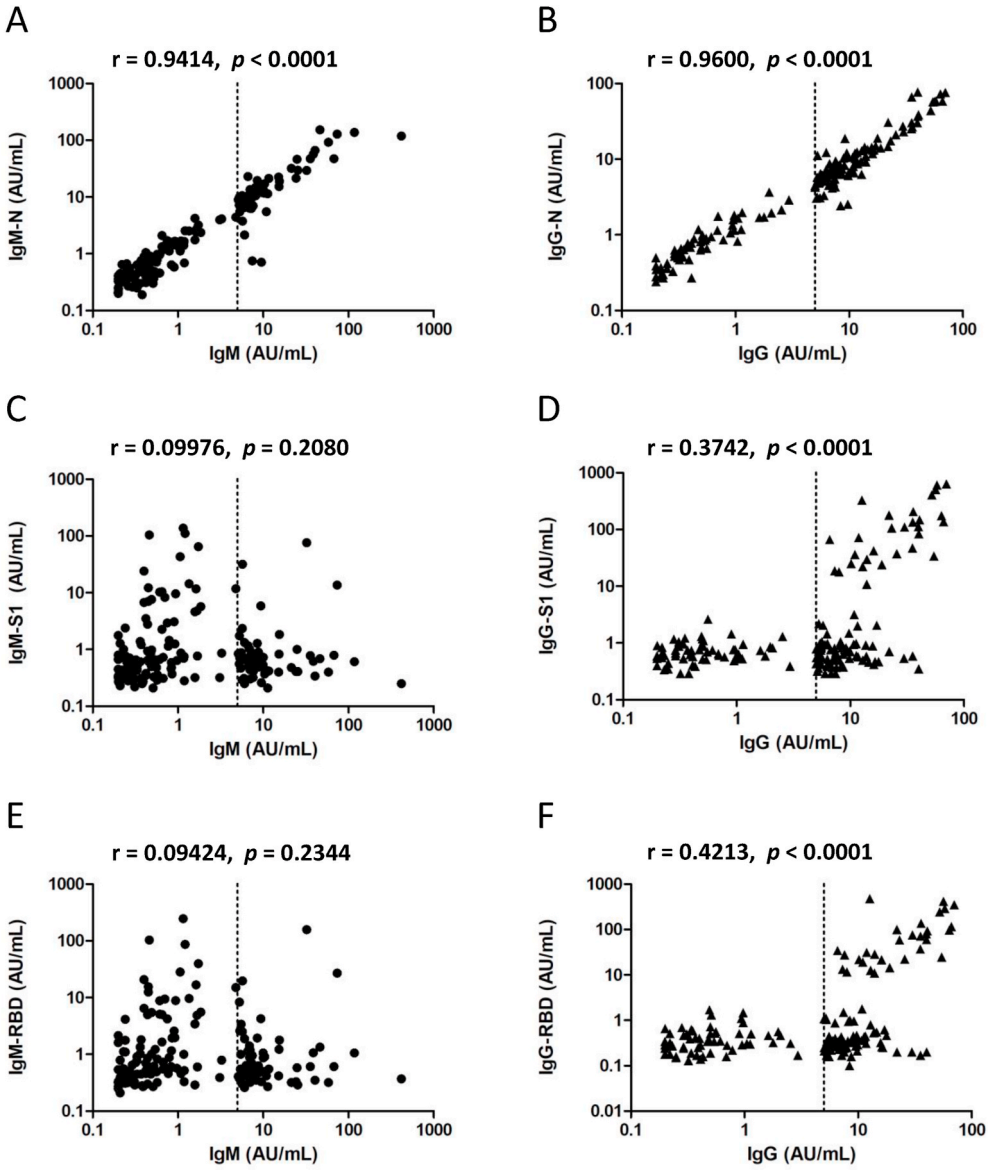

Fig. 1. Correlations of YHLO IgM or IgG titers and the reactivity to the N, S1, and RBP proteinsPlot
of data from each participant. IgM-N, IgM-S1, and IgM-RBD are plotted with YHLO IgM (A, C, and E) and IgG-N, IgG-S1, and IgG-RBD with YHLO IgG (B, D, and F).

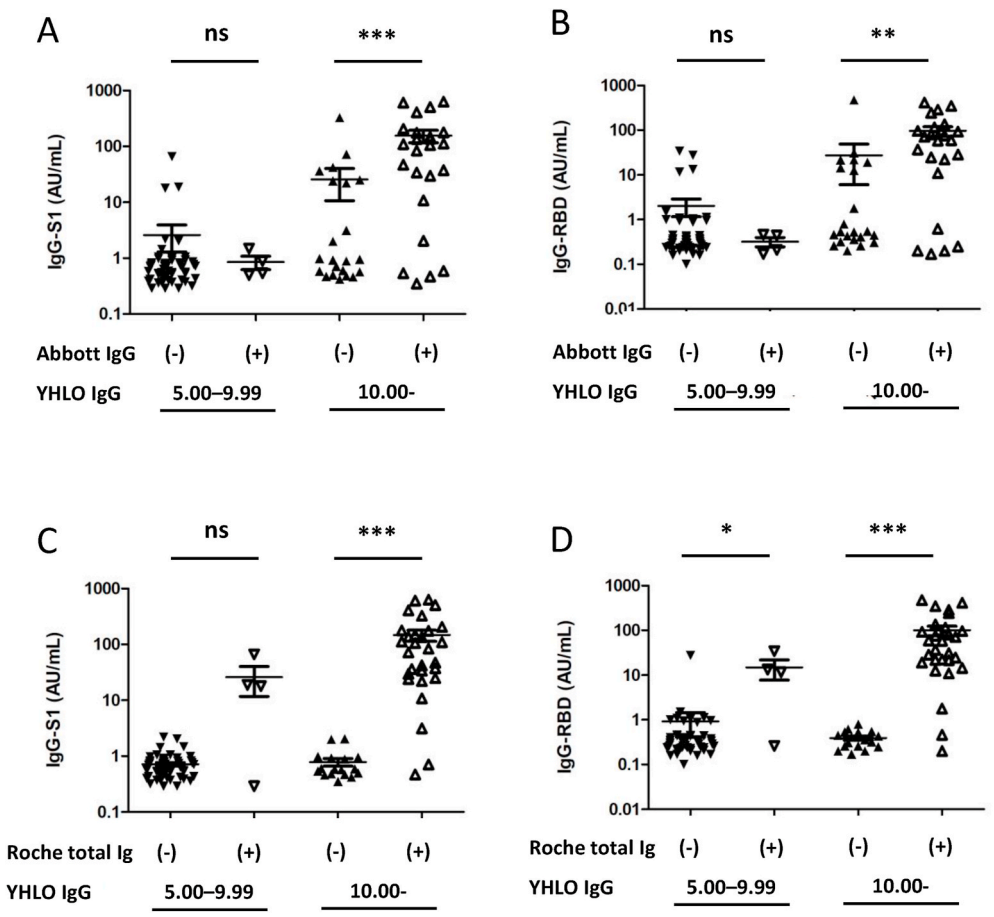

Fig. 2. Samples with increased YHLO IgG are classified according to the results of Abbott IgG or Roche total Ig.
IgG-S1 (A) and IgG-RBD (B) are plotted on the results of YHLO IgG and Abbott IgG, and IgG-S1 (C) and IgG-RBD (D) on the results of YHLO IgG and Roche total Ig. **: $p < 0.01$, ***: $p < 0.001$.

a 1.0 COI or more titers of the Roche total Ig and 1.4 index (S/C) or more of the Abbott IgG as positive results.

Among the samples with increased YHLO IgM, one (1.64%) was positive for Roche total Ig, and two (3.28%) were positive for Abbott IgG, all of which also showed increased YHLO IgG. Regarding YHLO IgG in 47 samples with 10.0 or more AU/mL titers of YHLO IgG, 29 samples (61.70%) showed positive results for Roche total Ig, and 25 samples (53.19%) for Abbott IgG. Between 5.0 AU/mL and 10.0 AU/mL, 57 samples with YHLO IgG titers still included 4 samples (7.02%) with positive Roche total Ig and 4 samples (7.02%) with positive Abbott IgG. Abbott IgG and Roche total Ig correlated well with YHLO IgG (r = 0.6588 and r = 0.5713, respectively) (Supplementary Figs. 1A and 1B); however correlation coefficient of Roche total Ig was smaller than that of Abbott IgG. Randomly selected 1204 samples from 9876 samples with YHLO IgM and IgG titers less than 5.0 AU/mL revealed 1 (0.08%) positive Roche total Ig and 1 (0.08%) positive Abbott IgG (Table 2).

3.3. YHLO IgM and IgG reactivity to N, S1, and RBD proteins

We subsequently measured the reactivity to N, S1, and RBD proteins of the same 163 samples. YHLO IgM titers and the titers of IgM against N protein (IgM-N), and YHLO IgG titers and titers of IgG against N protein (IgG-N) were strongly correlated (r = 0.9414 and r = 0.9600, respectively) (Fig. 1 A and B). Conversely, there was no correlation between YHLO IgM titers and IgM-S1 or IgM-RBD (Fig. 1C and E), and there was a significant but relatively small correlation between YHLO IgG and IgG-S1 or IgG-RBD (r = 0.3742 and r = 0.4213, respectively) (Fig. 1 D and F). Interestingly, there seemed to be two subgroups within the increased YHLO IgG category according to the IgG-S1 or IgG-RBD titers (Fig. 1 D and F); the high (more than 10.0 AU/mL) IgG-S1 or IgG-RBD group and low (less than 4.0 AU/mL) IgG-S1 or IgG-RBD group. There was also a strong correlation between IgM-S1 and IgM-RBD and IgG-S1 and IgG-RBD (Supplementary Fig. 2 A and B).

We divided samples with increased YHLO IgG into two groups using the results of Roche total Ig or Abbott IgG. When divided with Abbott IgG results, the positive Abbott IgG group showed significantly increased IgG-S1 and IgG-RBD values in samples with 10.0 AU/mL or more titers of YHLO IgG (Fig. 2 A and B). When the cutoff values of IgG-S1 and IgG-RBD were set at 10.0 AU/mL, the sensitivities of Abbott IgG in predicting positive IgG-S1 and IgG-RBD was 65.5% and 63.3%, respectively, and

Table 3
Two samples with YHLO IgM and IgG negative but Roche total Ig or Abbott IgG-positive results.

No.	YHLO IgM (AU/mL)	YHLO IgG (AU/mL)	Roche total Ig (COI)	Abbott IgG (index (S/C))	IgM-N (AU/mL)	IgG-N (AU/mL)	IgA-N (AU/mL)	IgM-S1 (AU/mL)	IgG-S1 (AU/mL)	IgA-S1 (AU/mL)	IgM-RBD (AU/mL)	IgG-RBD (AU/mL)	IgA-RBD (AU/mL)
1	0.24	0.38	0	2.07	0.29	0.66	0.38	0.33	0.46	0.26	0.44	0.31	0.29
2	0.49	2.05	4.7	0.19	0.5	1.6	5.21	0.49	2.19	0.52	0.48	1.3	0.74

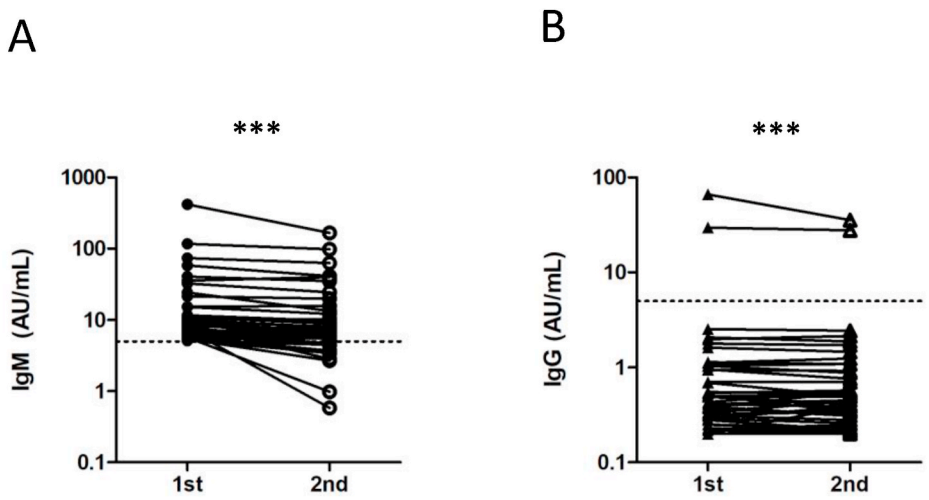

Fig. 3. Follow-up blood collection in samples with increased YHLO IgM. YHLO IgM (A) and YHLO IgG (B) were measured after the follow-up. ***: $p < 0.001$.

the specificities were 87.8% and 87.7%, respectively, in the samples with elevated YHLO IgG (≥5.0 AU/mL). When classified according to Roche total Ig results, the positive Roche total Ig group had significantly increased IgG-S1 and IgG-RBD values in samples with 10.0 AU/mL or more titers of YHLO IgG (0.79 ± 0.12 AU/mL vs. 147.2 ± 33.3 AU/mL and 0.39 ± 0.04 AU/mL and 100.2 ± 23.8 AU/mL) (Fig. 2C and D). When the cutoff values of IgG-S1 and IgG-RBD were set again as 10.0 AU/mL, the sensitivity and specificity of Roche total Ig for IgG-S1 results were 100.0% and 94.6%, and those for IgG-RBD results were 96.7% and 94.5% in the increased YHLO IgG (≥5.0 AU/mL) group.

Table 3 shows the reactivity to N, S1, and RBD proteins of the two samples described in Table 2 in which YHLO IgM and IgG were not increased although the results of Roche total Ig or Abbott IgG remained positive. One sample with a positive Abbott IgG showed no reactivity to any proteins at all, and one with the positive Roche total Ig showed small reactivity to the S1 protein; however, it contained mainly IgA.

3.4. Follow-up of participants with increased YHLO IgM titers

To investigate whether YHLO IgG titers would increase later in participants with increased YHLO IgM titers, they were asked to provide another blood sample at least three weeks after the first collection. Forty-four (72.1%) participants agreed, and we collected their blood samples. The mean number of days between the first and second blood collection was 55.6 ± 3.7 days. The YHLO IgM titers significantly decreased the second time (25.5 ± 9.7 AU/mL vs. 16.3 ± 4.4 AU/mL, $p < 0.0001$) (Fig. 3A). The YHLO IgG titers also slightly but significantly decreased (2.8 ± 1.6 AU/mL vs. 2.1 ± 1.0 AU/mL, $p < 0.0001$), and more importantly, no second sample became positive for YHLO IgG, except for the two participants who had initially been positive for YHLO IgG the first time (Fig. 3B).

3.5. Participants with a past history of COVID-19

Sixteen participants (0.16%) had a past history of COVID-19. Their characteristics and antibody titers are summarized in Table 4. Ten samples showed increased YHLO IgG of 5.0 AU/mL or more. They all had positive Roche total Ig and increased IgG-S1 and IgG-RBD, although Abbott IgG was positive only in 7 of 10. The other six samples without increased YHLO IgG were also negative for Roche total Ig and Abbott IgG, which had been additionally measured in five samples. One blood sample was collected on the same day of the diagnosis, and another more than six months later.

3.6. Reproducibility of the results of YHLO IgM and IgG

The remaining serum was stored at −80 °C, and YHLO IgM and IgG were measured again to see the reproducibility in 39 samples with increased IgM and 63 samples with increased IgG. The rates of change were plotted and are shown in Supplementary Fig. 3. The rate of change was −15.3 ± 4.0% for YHLO IgM and −4.0 ± 1.2% for YHLO IgG. Nine samples (23.1%) showed an IgM decrease of more than 30%, although no samples showed the same level of decrease in IgG.

4. Discussion

In the present study we extracted 163 samples with increased titers of iFlash-SARS-CoV-2 IgM or IgG (YHLO IgG) among 10,039 asymptomatic healthy volunteers, and performed further investigations using Roche total Ig, Abbott IgG, and IgM and IgG reactivity to N, S1, and RBD proteins.

Although YHLO IgM correlated well with IgM-N, it was not correlated with IgM-S1 or IgM-RBD. There were very few cases of increased IgM-S1 or IgM-RBD in YHLO IgM-positive cases, though YHLO IgG-positive cases often had IgM reactivity to S1 or RBD without an increase of YHLO IgM. Furthermore, no increase in YHLO IgG was observed in the paired sera of all the samples that we could follow-up, including the cases with elevated IgM-S1. The serum level of IgM generally increases ahead of that of IgG; however, in symptomatic patients with SARS-CoV-2 infection, it has been reported that the rise of IgM occurred almost concurrently with that of IgG, or sometimes IgG increased ahead of IgM [10]. These results made us speculate that although the elevated YHLO IgM might reflect the very early stages of infection, most cases could be false positives. It might be explained by the low positive predictive value due to the relatively low prevalence since this study targeted asymptomatic individuals in Japan. There is also the possibility of false positives due to cross-reaction with the seasonal coronavirus strains [10]. Therefore, YHLO IgM might not be suitable for the screening of healthy population in areas with a low prevalence of COVID-19, including Japan as of 2020.

As for YHLO IgG, it was also well correlated with IgG-N, and it was interesting that YHLO IgG elevated cases seemed to comprise a mixture of IgG-S1 elevated and non-elevated cases (and IgG-RBD elevated and non-elevated cases). The fact that those with a history of COVID-19 with elevated YHLO IgG showed an increased IgG-S1 without any exceptions made us speculate that an elevated YHLO IgG without an increased IgG-S1 might be a false positive, although it is impossible to prove this subgroup had no history of COVID-19.

We also measured Roche total Ig and Abbott IgG, which are run on a

Table 4
Participants with a past history of COVID-19.

age (years)	Sex	days after diagnosis	Diagnostic method		YHLO IgM (AU/mL)	YHLO IgG (AU/mL)	Roche total Ig (COI)	Abbott IgG (index (S/C))	IgM- N (AU/mL)	IgG- N (AU/mL)	IgA- N (AU/mL)	IgM- S1 (AU/mL)	IgG- S1 (AU/mL)	IgA- S1 (AU/mL)	IgM-RBD (AU/mL)	IgG-RBD (AU/mL)	IgA-RBD (AU/mL)
27	F	0			0.2	0.2	0	0.02									
48	F	19	antigen		0.2	0.46	0	0.07									
18	M	35	PCR	+	1.06	40.61	33.5	3.85	1.12	38.76	3.74	43.02	148.05	148.1	28.3	93.32	139.33
40	F	61	PCR	+	0.44	13.96	6.1	1.6	0.62	11	1.63	2.78	10.87	4.27	4.99	10.91	4.17
42	F	approx. 80			0.54	0.19	0	0.01									
26	F	90		+	0.9	12.85	9.3	0.96	0.58	6.28	0.64	3.09	22.07	1.09	2.58	12.65	1.16
33	F	approx. 100		+	0.7	63.39	89.8	3.42	1.39	72.48	1.33	8.3	176.46	10.9	9.47	96.81	10.84
23	M	104	PCR	+	0.4	30	65.8	2.31	0.53	23.13	0.61	6.71	110.41	5.42	6.47	76.11	5.87
36	F	approx. 130			0.26	0.2	0	0.01									
67	M	approx. 130	PCR	+	0.37	70.2	193	4.72	0.76	75.97	31.27	1.22	632.86	42.55	1.81	348.78	47.75
47	M	132	PCR	+	0.4	56.36	183	4.29	0.89	57.8	1.58	24.01	508.59	4.96	20.75	415.34	5.23
24	F	132	PCR	+	0.45	6.6	5.4	0.85	0.65	4.54	0.72	7.05	66.57	2.69	12.56	34.17	2.05
28	F	168			0.3	0.2	0	0.04									
56	M	169	PCR	+	0.75	21.95	64	1.62	1.71	14.67	8.52	2.94	179.15	10.06	4.21	99.48	12.17
56	F	178	PCR	+	1.63	11.86	25.1	0.83	2.42	8.75	0.85	11.72	71.93	1.43	16.74	31.12	1.42
31	M	195			0.23	1.48	0	0.02									

commercial basis in many countries including Japan. YHLO IgG and Abbott IgG were well correlated, and Abbott brought us little additional information when used with YHLO IgG. At a cutoff value of 5.0 AU/mL, the elevated YHLO IgG cases included almost all cases with positive Abbott IgG. Conversely, the combined use with Roche total Ig may efficiently improve the YHLO IgG test. This is because it could select IgG-S1 or IgG-RBD elevated cases with high sensitivity and specificity, whereas the single use of Roche total Ig may not be suitable because it measures various kinds of immunoglobulins. It was interesting that Roche total Ig, which reacts to the N protein, could be a good marker of the reactivity to the S1 or RBD proteins when used with YHLO IgG.

Some samples with a past history of COVID-19 showed no increase of YHLO IgG; however, it should be emphasized that the results of Roche total Ig or Abbott IgG were also negative in these cases. Previous reports have revealed that IgG against SARS-CoV-2 gradually declined and sometimes resulted in a seronegative result six months after infection [17]. With the pandemic of the new coronavirus lasting for over a year, caution should be applied in the interpretation of these findings.

Both YHLO IgM and IgG had a reproducibility of results after the cryopreservation, although some of the YHLO IgM positive cases turned clearly negative. There are several limitations in this study. First, members of the University of Tokyo do not always reflect the population of Tokyo or Japan. Second, because we did not perform SARS-COV-2 PCR tests or antigen tests, it is not possible to evaluate whether it is truly positive or negative. This is a universal problem of seroepidemiological studies; however, we performed follow-up blood collection in some cases, and asked about a past history of COVID-19 using the anonymous online questionnaire in order to solve at least a part of this problem.

In conclusion, attention must be given to the interpretation of the results of YHLO IgM and IgG in SARS-CoV-2 seroepidemiological studies of asymptomatic subjects, especially for YHLO IgM. The combined use of YHLO IgG and Roche total Ig could produce more reliable results in seroepidemiological studies of SARS-CoV-2.

Author contributions

AM, TH, KH, MK, YY, and SY conceived and designed the study. AM, TH, KH, YI, and SY coordinated the study. AM, TH, KH, YI, MS, TI, MS, RT, TU, NE, NN, YM, SO, ST, KI, AY, and SY collected informed consent from the study participants. RY and YN managed the blood sample collection and performed the measurements of the reactivity to specific proteins. AM and TH wrote the manuscript. All authors have read, edited, and approved the final manuscript.

Funding

This work was supported by the University of Tokyo, Promoting practical use of measures against coronavirus disease 2019 (COVID-19).

Declaration of competing interest

The authors declare that they have no conflicts of interest relevant to this study.

Acknowledgment

We appreciate the Murakami Foundation's donation of the iFlash 3000 device to The University of Tokyo Hospital.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2021.11.020.

References

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382(8):727–33.

[2] Ministry of Health Labor and Welfare. About Coronavirus Disease 2019 (COVID-19). https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000164708_00001.html.

[3] Yuce M, Filiztekin E, Ozkaya KG. COVID-19 diagnosis -A review of current methods. Biosens Bioelectron 2021;172:112752.

[4] Boger B, Fachi MM, Vilhena RO, Cobre AF, Tonin FS, Pontarolo R. Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. Am J Infect Control 2021;49(1):21–9.

[5] Nakano Y, Kurano M, Morita Y, Shimura T, Yokoyama R, Qian C, et al. Time course of the sensitivity and specificity of anti-SARS-CoV-2 IgM and IgG antibodies for symptomatic COVID-19 in Japan. Sci Rep 2021;11(1):2776.

[6] Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. Jama 2020;323(22):2249–51.

[7] Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. Ann Intern Med 2020;173(5):362–7.

[8] Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory diagnosis of COVID-19: current issues and challenges. J Clin Microbiol 2020;58(6).

[9] Ma H, Zeng W, He H, Zhao D, Jiang D, Zhou P, et al. Serum IgA, IgM, and IgG responses in COVID-19. Cell Mol Immunol 2020;17(7):773–5.

[10] Yokoyama R, Kurano M, Morita Y, Shimura T, Nakano Y, Qian C, et al. Validation of a new automated chemiluminescent anti-SARS-CoV-2 IgM and IgG antibody assay system detecting both N and S proteins in Japan. PLoS One 2021;16(3):e0247711.

[11] Chan CW, Parker K, Tesic V, Baldwin A, Tang NY, van Wijk XMR, et al. Analytical and Clinical Evaluation of the Automated Elecsys Anti-SARS-CoV-2 Antibody Assay on the Roche cobas e602 Analyzer. Am J Clin Pathol 2020;154(5):620–6.

[12] Meschi S, Colavita F, Bordi L, Matusali G, Lapa D, Amendola A, et al. Performance evaluation of Abbott ARCHITECT SARS-CoV-2 IgG immunoassay in comparison with indirect immunofluorescence and virus microneutralization test. J Clin Virol 2020;129:104539.

[13] Kontou PI, Braliou GG, Dimou NL, Nikolopoulos G, Bagos PG. Antibody tests in detecting SARS-CoV-2 infection: a meta-analysis. Diagnostics 2020;10(5).

[14] Yoshiyama T, Saito Y, Masuda K, Nakanishi Y, Kido Y, Uchimura K, et al. Prevalence of SARS-CoV-2-specific antibodies, Japan, June 2020. Emerg Infect Dis 2021;27(2):628–31.

[15] Antibody prevalence survey (in Japanese). https://www.mhlw.go.jp/content/000734482.pdf.

[16] Mitani A, Hamada K, Yoshikawa N, Morita Y, Horie T, Inoue Y, et al. Epidemiological study using IgM and IgG antibody titers against SARS-CoV-2 in the university of Tokyo, Japan (UT-CATS). J Infect Chemother 2021;27(9):1342–9.

[17] Harvey RA, Rassen JA, Kabelac CA, Turenne W, Leonard S, Klesh R, et al. Association of SARS-CoV-2 seropositive antibody test with risk of future infection. JAMA Intern Med 2021;181(5):672–9.