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Evaluation of ichroma™ COVID-19 interferon gamma release assay for detection of vaccine-induced immunity in healthcare workers

https://doi.org/10.1515/cclm-2022-0914
Received September 14, 2022; accepted November 29, 2022; published online December 8, 2022

Abstract

Objectives: We compared the performance of a new interferon gamma release assay (IGRA) format assay, the ichroma™ COVID-19 IGRA (IGRA-SARS), with that of the widely used QuantiFERON SARS-CoV-2 ELISA kit (QFN-SARS) in vaccinated healthcare workers (HCWs). Additionally, we analyzed the long-term changes in IGRA results after the final vaccine dose.

Methods: A total of 383 specimens from 281 HCWs were enrolled in this study, and the results of SARS-IGRA and QFN-SARS assays were compared. In addition, we performed the receive operator curve analysis to estimate the optimal cut-off value for IGRA-SARS.

Results: For all specimens, IGRA-SARS and QFN-SARS showed 75.7% and 64.2% of the positive results, respectively. The absolute agreement between IGRA-SARS and QFN-SARS was 80.0%, and the Fleiss' κ value was 0.525, indicating moderate agreement. ROC curve analysis of the IGRA-SARS results showed a cut-off value of >0.254 IU/mL, which was consistent with the manufacturer's specifications. The positive rates of both IGRA assays decreased significantly after a postvaccination period of 6 months.

Conclusions: IGRA-SARS showed acceptable performance in the detection of vaccine-induced immunity against COVID-19; however, harmonization of IGRA assays has not yet been achieved. Additionally, the significant decline of positive rates of IGRA after the last vaccination would support the necessity of booster vaccination after a postvaccination period of 6 months.

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Keywords: COVID-19; evaluation; interferon gamma release assay; SARS-CoV-2.

Introduction

Since the rapid spread of the novel coronavirus, which was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), from the People's Republic of China [1], there has been a global race to develop and deploy vaccines against SARS-CoV-2 [2]. Alongside ongoing COVID-19 vaccinations, there has been significant public interest in evaluating the immune status of individuals against SARS-CoV-2. Despite the development of assays for the detection of antibody against SARS-CoV-2, it is still unclear if the presence of SARS-CoV-2 antibodies is protective against SARS-CoV-2 infection or if it can serve as a predictor of the clinical course of COVID-19 [3].

The immune response against pathogens involves both antibody production and T-cell response induction in patients. The interferon gamma (IFN-γ) release assay (IGRA) is among the most effective tools to measure the stimulated response of antigen-specific T cells to pathogen-specific peptides [4]. Notably, IGRA format assays have been widely evaluated for various infectious diseases and have shown acceptable performance in the clinical setting [5–8]; therefore, IGRA with SARS-CoV-2-specific peptides has become a good alternative to antibody assays for monitoring an immune response to SARS-CoV-2. Many IGRA technique-based assays were developed in the early days of the COVID-19 pandemic, and the clinical performance of these kits was globally evaluated. According to recent researches, IGRA against SARS-CoV-2 is expected to investigate infection severity in hospitalized COVID-19 patients, clinical prognosis in intensive care unit patients, and T-cell immune response following COVID-19 vaccination [4, 5, 9–13].

Recently, a new IGRA format-based assay, the ichroma™ COVID-19 IGRA (IGRA-SARS; Boditech Med, Gangwon-do, Republic of Korea), has been commercially introduced. The principle of IGRA-SARS includes stimulating T-cells using the SARS-CoV-2-specific antigen and measuring the IFN-γ released by antigen-specific T-cell.

These procedures are almost the same as other IGRAs currently available; however, IGRA-SARS differs from other assays in its measurement method of IFN-γ: IGRA-SARS uses a compact automated analyzer to quantify IFN-γ levels, whereas other IGRAs use a manual ELISA kit. In addition, the SARS-CoV-2-specific peptides used in IGRA vary among manufacturers [14, 15].

In this study, we enrolled healthcare workers (HCWs) who were vaccinated with at least two doses against SARS-CoV-2 and compared the analytical performance of IGRA-SARS with that of QuantiFERON SARS-CoV-2 ELISA kit (QFN-SARS; QIAGEN, MD, USA) for detection of immunity against SARS-CoV-2. In addition, we analyzed the long-term changes in IGRA results after the final vaccine dose.

Materials and methods

Study participants

This study was performed from November 2021 to March 2022 at the Chung-Ang University Hospital (Seoul, Republic of Korea). The inclusion criteria were HCWs who had received at least two vaccine doses against COVID-19 irrespective of the manufacturer of the vaccine (AstraZeneca COVID-19 vaccine, Pfizer-BioNTech COVID-19 vaccine, or Moderna COVID-19 Vaccine). HCWs with a history of COVID-19 infection were excluded. During the study period, a total of 281 HCWs were involved in this study, and they were examined by IGRA-SARS and QFN-SARS. Additionally, to assess the optimal cut-off value for IGRA-SARS, we enrolled 48 healthy participants (non-HCWs) who neither had been exposed to SARS-CoV-2 nor had received the COVID-19 vaccination. This study was approved by the Institutional Review Board (IRB) of Chung-Ang University Hospital (IRB No. 2108-007-473), and all participants provided written informed consent.

ichroma™ COVID-19 IGRA (IGRA-SARS)

IGRA-SARS is a fluorescence Immunoassay (FIA) for the detection of the IFN-γ released in response to *in vitro* stimulation by SARS-CoV-2-specific antigen in human whole blood. The procedure of IGRA-SARS comprised two stages: the first stage included incubation of blood from each tube, and the second stage involved the measurement of plasma IFN-γ by a lateral flow immunoassay. Herein, 1 mL of whole blood was collected into each of IGRA-SARS tubes: Nil tube, COVID-19 antigen tube, and mitogen tube. Immediately, these tubes were gently shaken to ensure that the additive and blood had mixed well and then transferred to a 37 °C incubator. After 16 h of incubation, the tubes were centrifuged at 2500 *g* for 15 min, and the plasma was separated. Then, IFN-γ levels (IU/mL) for each tube were quantified using ichroma™ II (Boditech Med), which is a compact, fluorescence-based lateral flow immune-analyzer that measures the concentration of the analyte. The IFN-γ results of the Nil tube, COVID-19 antigen tube, and mitogen tube are referred to as the $Nil_{IGRA\text{-}SARS}$ value, $AG_{IGRA\text{-}SARS}$ value, and $Mit_{IGRA\text{-}SARS}$. The results were interpreted as per the manufacturer's specifications (Table 1).

Table 1: Interpretation of IGRA-SARS (A) and QFN-SARS (B) test results.

(A) Interpretation of IGRA-SARS results

$Nil_{IGRA\text{-}SARS}$, IU/mL	$AG_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$, IU/mL	$Mit_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$, IU/mL	Result
≤8.0	<0.25	≥0.5	Non-reactive
≤8.0	≥0.25 and <25% nil	≥0.5	Non-reactive
≤8.0	≥0.25 and ≥25% nil	Any	Reactive
≤8.0	<0.25	<0.5	Indeterminate
≤8.0	≥0.25 and <25% nil	<0.5	Indeterminate
>8.0	Any	Any	Indeterminate

(B) Interpretation of QFN-SARS results

$Nil_{QFN\text{-}SARS}$, IU/mL	$AG1_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$, IU/mL	$AG2_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$, IU/mL	$Mit_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$, IU/mL	Result
≤8.0	<0.15 or ≥0.15 and <25% nil	<0.15 or ≥0.15 and <25% nil	≥0.50	Non-reactive
≤8.0	≥0.15 and ≥25% nil	Any	Any	Reactive
≤8.0	Any	≥0.15 and ≥25% nil	Any	Reactive
≤8.0	<0.15 or ≥0.15 and <25% nil	<0.15 or ≥0.15 and <25% nil	<0.50	Indeterminate
≥8.0	Any	Any	Any	Indeterminate

QuantiFERON SARS-CoV-2 ELISA kit (QFN-SARS)

The procedure of QFN-SARS is almost analogous to that of IGRA-SARS; however, there are difference in the number of blood tubes and the IFN-γ measurement methods. Whole blood is collected into four QuantiFERON blood collection tubes, which includes the nil tube, mitogen tube, and two S peptide tubes (Ag1 and Ag2). After a 16–24-h incubation at 37 °C, the tubes are centrifuged, and the amount of IFN-γ (IU/mL) is measured by ELISA using the plasma. The plasma levels of IFN-γ for Ag1, Ag2, Nil, and mitogen tubes are referred as the $AG1_{QFN\text{-}SARS}$, $AG2_{QFN\text{-}SARS}$, $Nil_{QFN\text{-}SARS}$, and $Mit_{QFN\text{-}SARS}$, respectively. QFN-SARS results are interpreted as per the manufacturer's specifications (Table 1).

Statistics

For assessing the agreement between IGRA-SARS and QFN-SARS, Fleiss' kappa coefficients were calculated and assessed according to

the following criteria: 0.81–1.00 for almost perfect agreement, 0.61–0.80 for substantial agreement, 0.41–0.60 for moderate agreement, 0.21–0.40 for fair agreement, 0.00–0.20 for slight agreement, and <0.00 for poor agreement [16]. Deming regression analysis was used to compare the antigen-stimulated IFN-γ levels minus Nil values from IGRA-SARS and QFN-SARS. To estimate the optimal cut-off value of IGRA-SARS, we performed receive operator curve (ROC) analysis using IGRA-SARS results for HCW participants and healthy participants. In addition, to analyze the changes of IGRA positive rates in accordance with the day after the final vaccination, we divided the results of IGRA assays into five groups according to the number of days passed since the last vaccine dose: <30 days, 30–59 days, 60–89 days, 90–179 days, and ≥180 days. Then, Pearson's chi-square test with post-hoc analysis was used to compare the positive rates of IGRA-SARS and QFN-SARS in accordance with the number of days passed since the final vaccine dose. A p-value of <0.05 was considered significant. All statistical analyses were performed with R version 4.0.3 (http://www.R-project.org/).

Results

Participant characteristics

A total of 383 specimens from 281 HCWs were included in this study (Table 2). The median age of participants was 43 years [interquartile range (IQR), 34–51], and 70.0% of the participants were women. All HCW participants were vaccinated twice [booster(–) group] or thrice [booster(+) group], and the median time period passed since vaccination was 282 days (IQR, 259–287) since the 1st vaccination and 208 days (IQR, 185–257) since the 2nd vaccination. Overall, 269 specimens (70.2%) were collected after the COVID-19 vaccine booster shot.

Table 2: Baseline characteristics of the 281 healthcare workers from whom 383 specimens were acquired.

	Total specimens (n=383)		Booster(–) specimens (n=114)		Booster(+) specimens (n=269)	
Age, median (IQR), years	43	(34–51)	40	(30–50)	43	(36–51)
Sex, male (%)	115	(30.0)	39	(34.2)	76	(28.3)
Days after vaccination (IQR)						
1st dose	282	(259–287)	224	(172–265)	285	(279–306)
2nd dose	208	(185–257)	158	(112–189)	209	(207–265)
Booster shots					22	(20–84)

IQR, interquartile range.

Comparison results between IGRA-SARS and QFN-SARS

The qualitative comparisons results are shown in Table 3. For all specimens, IGRA-SARS showed 75.7% positivity, which was a value slightly higher than that for QFN-SARS, which showed 64.2% positivity. The absolute agreement between IGRA-SARS and QFN-SARS was 80.0% (306/383), and the Fleiss' κ value was 0.525, which was interpreted as moderate agreement. When considering the booster shot, the positivity rates of IGRA-SARS and QFN-SARS were 62.3 and 44.7% for booster(–) specimens and 81.4 and 72.5% for booster(+) specimens, respectively. The absolute agreements for booster(–) and booster(+) specimens were 72.8% (73/114) and 82.9% (223/269), respectively, between IGRA-SARS and QFN-SARS qualitative results. The Fleiss' κ values were 0.458 for booster(–) specimens and 0.521 for booster(+) specimens, and these results indicated moderate agreement between the two groups.

The results of Deming regression analysis between the antigen-stimulated IFN-γ levels minus Nil values are shown in Figure 1. When comparing the antigen-stimulated IFN-γ levels minus Nil values, $AG_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$ values from IGRA-SARS were consistently higher than $AG1_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ values and $AG2_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ values from QFN-SARS. The slopes, intercepts, and correlation coefficients were 0.334, −0.127, and 0.7104 for a comparison between $AG_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$ values and $AG1_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ values, respectively. Similarly, these values were 0.488, −0.034, and 0.6591 for a comparison between the $AG_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$ values and $AG2_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ values, respectively.

Optimal cut-off evaluation in IGRA-SARS

To determine an optimal cut-off value for IGRA-SARS, an ROC analysis was performed based on the IGRA-SARS results of 383 HCW specimens and 48 specimens from healthy non-HCWs. All samples obtained from non-HCWs showed negative results in the IGRA-SARS. The AUC value was 0.9249 according to the Youden index, and the estimated cut-off value for COVID-19 vaccination positivity was 0.254 IU/mL with a sensitivity of 79.3% and specificity of 100% (Figure 2). This optimal cut-off value was the same as the value provided in manufacturer's specifications

Table 3: Qualitative comparisons between IGRA-SARS and QFN-SARS in total specimens (A), booster(–) specimens (B), and booster(+) specimens (C).

(A) Total specimens (n=383)											
		QFN-SARS								**Fleiss' kappa**	
		Positive		**Negative**		**Indeterminate**		**Total**			
IGRA-SARS	Positive	230	(60.1)	59	(15.4)	1	(0.3)	290	(75.7)	0.525	(0.426–0.624)
	Negative	14	(3.7)	76	(19.8)	0	(0.0)	90	(23.5)		
	Indeterminate	2	(0.5)	1	(0.3)	0	(0.0)	3	(0.8)		
	Total	246	(64.2)	136	(35.5)	1	(0.3)				
(B) Booster(–) specimens (n=114)											
		QFN-SARS								**Fleiss' kappa**	
		Positive		**Negative**		**Indeterminate**		**Total**			
IGRA-SARS	Positive	46	(40.4)	25	(21.9)	0	(0.0)	71	(62.3)	0.458	(0.277–0.639)
	Negative	5	(4.4)	37	(32.5)	0	(0.0)	42	(36.8)		
	Indeterminate	0	(0.0)	1	(0.9)	0	(0.0)	1	(0.9)		
	Total	51	(44.7)	63	(55.3)	0	(0.0)				
(C) Booster(+) specimens (n=269)											
		QFN-SARS								**Fleiss' kappa**	
		Positive		**Negative**		**Indeterminate**		**Total**			
IGRA-SARS	Positive	184	(68.4)	34	(12.6)	1	(0.4)	219	(81.4)	0.521	(0.404–0.639)
	Negative	9	(3.3)	39	(14.5)	0	(0.0)	48	(17.8)		
	Indeterminate	2	(0.7)	0	(0.0)	0	(0.0)	2	(0.7)		
	Total	195	(72.5)	73	(27.1)	1	(0.4)				

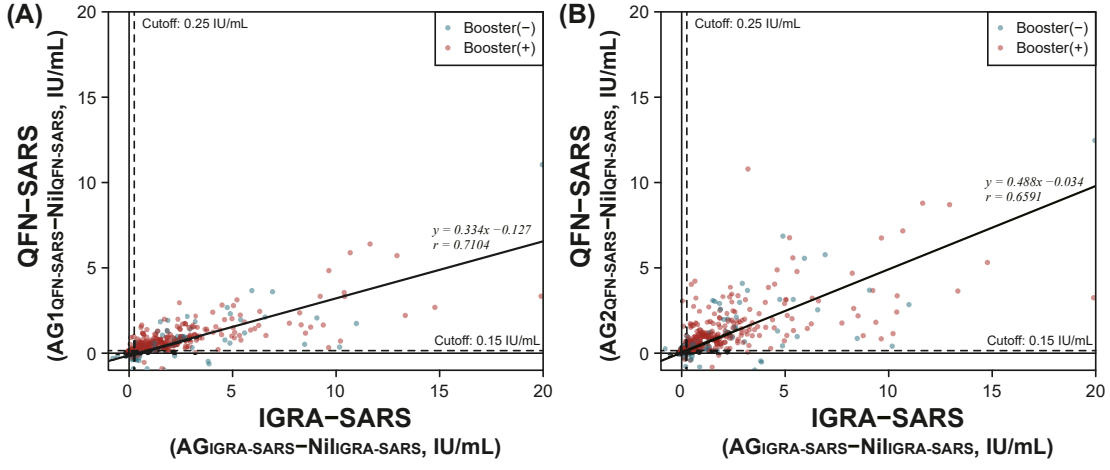

Figure 1: Scatter plots for $AG_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$ values from IGRA-SARS and for $AG1_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ (A) and $AG2_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ (B) values from QFN-SARS. The bold lines represent the Deming regression lines, and the dotted lines designate the cut-off levels of each assay.

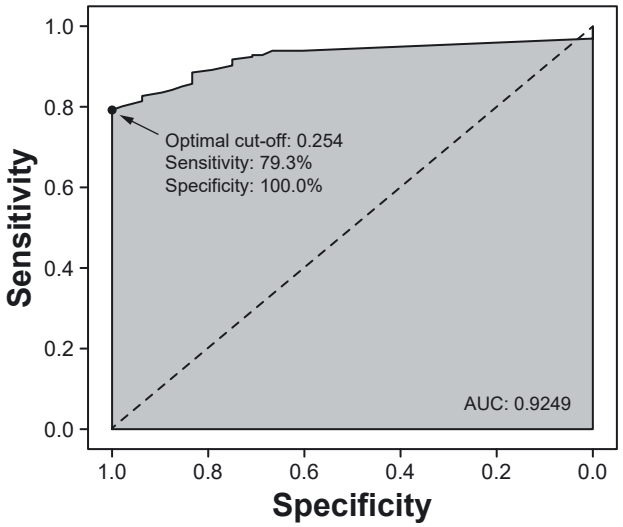

Figure 2: ROC curve of IGRA-SARS to assess T-cell response against SARS-CoV-2. AUC, area under the curve.

Positive rates of IGRA assays after the final vaccine dose

The positive rates of IGRA-SARS and QFN-SARS according to the number of days passed since the final vaccine dose are illustrated in Figure 3. As it was impossible to evaluate the immune response of patients with indeterminate results, we considered the indeterminate results as a negative result. Within 30 days of the final vaccination (<30 days group), the positive rates of IGRA-SARS and QFN-SARS were 87.8% (151/172) and 83.1% (143/172), respectively. Thereafter, the positive rates gradually decreased to 82.8% (30–59 days group, 24/29), 71.6% (60–89 days group, 53/74), 78.3% (90–179 days group, 36/46), and 51.6% (≥180 days, 32/62) for IGRA-SARS. Similarly, the positive rates were 55.2% (30–59 days group, 16/29), 54.1% (60–89 days group, 40/74), 60.9% (90–179 days group, 28/46), and 33.9% (≥180 days group, 21/62) for QFN-SARS. For both IGRA tests, there were significant differences in the positive rates between the groups in accordance with the day after the final vaccination. Especially, the positive rates of ≥180 days group were significantly lower than those of <30 days group in both IGRA assays, when the post-hoc analyses were performed.

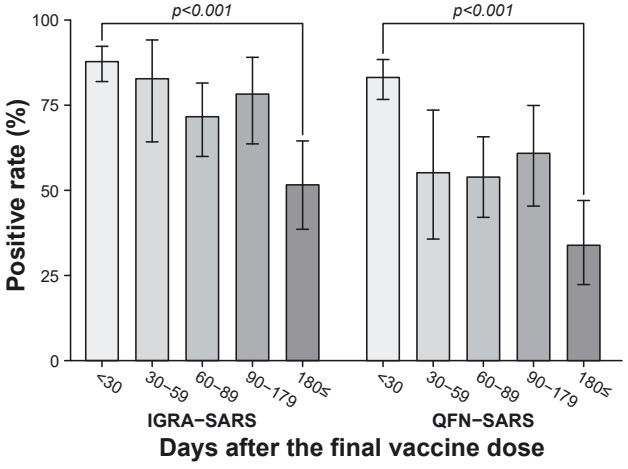

Figure 3: Positive rates of IGRA-SARS and QFN-SARS according to the number of days passed since the final vaccine dose. Error bars represent 95% confidence intervals. There were significant differences in the positive rates between the groups (p<0.001 for IGRA-SARS, and p<0.001 for QFN-SARS), and the positive rates of ≥180 days group were significantly lower than those of <30 days group in both IGRA assays.

Discussion

In this study, we evaluated the performance of IGRA-SARS, a newly developed IGRA against SARS-CoV-2, using clinical specimens from HCWs who were vaccinated with at least two doses. For the detection of vaccine-induced immunity, IGRA-SARS showed a superior positivity rate than QFN-SARS (75.7 vs. 64.2%). Because there is no reference method for evaluating immunity against SARS-CoV-2, we could not conclude which assay result is closer to the correct value. However, because all participants included in this study were vaccinated, we can assume that the assay with higher positivity more accurately reflect the immune status of T-cell response against SARS-CoV-2. The positivity of IGRA-SARS was higher than that of QFN-SARS, suggesting that the former more accurately reflects the immune status against SARS-CoV-2 than the latter. Additionally, the results of unvaccinated non-HCWs showed negative results, and the probability of a false-positive test would be very low.

When comparing the results of booster(–) and booster(+) groups, the difference in positivity rates between the two assays (IGRA-SARS vs. QFN-SARS) were higher in the booster(–) group (62.3 vs. 44.7%) than in the booster(+) group (81.4 vs. 72.5%). The median day after final vaccination in the booster(–) group was about 5 months; it was thought that the more sensitive assay would show a stronger vaccine-induced immunity even though the immune response against SARS-CoV-2 weakens with the passage of time after vaccination. In addition, the absolute agreement and Fleiss' kappa coefficient between the two assays was inferior in the booster(–) group than in the booster(+) group, and these results indicated that IGRA format assays could show discordant results if sufficient

immunity against SARS-CoV-2 is not maintained. Therefore, IGRA-SARS or QFN-SARS results for estimating vaccine-induced immunity against COVID-19 should be interpreted with great caution.

In Deming regression analysis, we found that stimulated IFN-γ levels minus Nil values were higher for IGRA-SARS than for QFN-SARS. $AG_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$ values from IGRA-SARS were approximately 2–3 times higher than $AG1_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ values and $AG2_{QFN\text{-}SARS}$ minus $Nil_{QFN\text{-}SARS}$ values from QFN-SARS, and the discordant qualitative results could be attributed to these differences. These differences could be due to differences in antigens that stimulate T-cells and the quantification method of IFN-γ [14, 15], and harmonization between IGRA format assays is considered necessary, particularly in the crucial components of these assays, such as stimulating antigens and IFN-γ measurement methods.

According to the manufacturer's specifications, a cut-off value of >0.25 IU/mL was suggested for $AG_{IGRA\text{-}SARS}$ minus $Nil_{IGRA\text{-}SARS}$. The use of IGRA format assays for detecting a latent TB infection has revealed several issues about the appropriateness of the cut-off value provided by the manufacturer [6, 17]. To validate the cut-off value for IGRA-SARS, we enrolled additional non-HCW participants who had no history of COVID-19 and no vaccination against SARS-CoV-2. In this study, the optimal cut-off value of >0.254 IU/mL was chosen based on the results of the ROC analysis, and when this cut-off value was adopted, the sensitivity and specificity of IGRA-SARS was estimated to be 79.3 and 100%, respectively. This optimal cut-off value was consistent with the manufacturer's specifications, and we demonstrated that the manufacturer-recommended cut-off was well established when screening for vaccine-induced immunity against SARS-CoV-2.

In the analysis of the positive rates according to the number of days passed since the final vaccine dose, the positive rates of two IGRA assays were over 80% in the specimens taken 30 days before the final vaccination. Then, the positive rates decreased to about 51.6% for IGRA-SARS and 33.9% for QFN-SARS. Recently, A Lasagna and colleagues reported that the positive rates of SARS-CoV-2 IGRA assay were 76.6% 3 week after third vaccination and 62.3% 6 months after third vaccination [18]. In agreement with this study, the positive results of IGRA-SARS and QFN-SARS showed decreasing trends after the final vaccine dose in the present study, and we found a significant decline in IGRA positive rates after a postvaccination period of 6 months. When considering the humoral response against SARS-CoV-2, Oliveira-Silva et al. showed that there was a decrease in the level of antibodies against SARS-CoV-2 after a postvaccination period of 6 months [19]. Taken together, the *in vitro* tests for both antigen-specific T cell response and humoral response would significantly decrease after a postvaccination period of 6 months. Although the severity of COVID-19 could not be fully predicted as a result of laboratory tests, these findings would strengthen the requirement of booster vaccination after a postvaccination period of 6 months [19–21].

This study has several limitations. First, we could not take into account the type of vaccine administered to HCWs. It is well known that the antigen used for each vaccine is different, which would influence the immune response against the synthetic peptides used in IGRA format assays. Second, because there is no reference method for detection of immunity against COVID-19, we could not evaluate the exact clinical performance of IGRA-SARS and QFN-SARS. Third, we enrolled the HCW participants anonymously, and we could not determine the factors influencing discordant results between IGRA-SARS and QFN-SARS.

In conclusion, a new IGRA format assay against SARS-CoV-2, IGRA-SARS, showed acceptable performance in the detection of vaccine-induced immunity against COVID-19. Its performance was superior to that of QFN-SARS, which is the most widely used assay in the same technical category. However, as there was no harmonization across the IGRA format assays, the discordance in results of various assays was inevitable, and the results should be interpreted with caution. Additionally, the decline of IGRA positive rates after the last vaccination would indicate a weakened antigen-specific T-cell immunity against SARS-CoV-2, thus emphasizing the necessity of booster vaccination after a postvaccination period of over 6 months.

Acknowledgments: We thank the Diagnostic Immunology department of the Chung-Ang University Hospital for their contribution to this study for a long time.

Research funding: This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2020R1A5A1018052). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions: Lee MK and Kim TH conceived the presented idea and supervised the findings of this work. Choi YJ and Kweon OJ performed the calculations and designed the figures. Lim YK wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version.

Competing interests: The authors declare that there is no conflict of interest regarding the publication of this article.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: This study was approved by the Institutional Review Board (IRB) of Chung-Ang University Hospital (IRB No.2108-007-473).
Data availability: The datasets generated during the current study are available from the corresponding author on reasonable request.

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