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Original Article

Performance analysis among multiple fully automated anti-SARS-CoV-2 antibody measurement reagents: A potential indicator for the correlation of protection in the antibody titer

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ABSTRACT

Background: To evaluate the performance of various reagents in automated analyzers for antibody detection against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: Using 100 serum samples from 100 individual patients diagnosed with SARS-CoV-2 infection, the precision, linearity, determination agreement, and correlation of five qualitative reagents (Elecsys Anti-SARS-CoV-2, ARCHITECT SARS-CoV-2 IgG, ARCHITECT SARS-CoV-2 IgM, Access SARS-CoV-2 IgM, and SARS-CoV-2 IgM) and four quantitative reagents (Elecsys Anti-SARS-CoV-2 S, ARCHITECT SARS-CoV-2 IgG II, Access SARS-CoV-2 IgG 1st IS, and SARS-COV-2 IgG S) were analyzed. A surrogate virus-neutralizing test (sVNT) kit was used to evaluate the measurement value of each quantitative reagent corresponding to the amount of neutralizing antibody, similar to that of patients in the late stage of infection.

Results: Precision and linearity were found to be sufficient for clinical use. Five discrepant samples were observed in the positive and negative judgments of the qualitative reagents for IgG, and one discrepant sample was observed in the qualitative reagent for IgM. Although the measurement values of the quantitative reagents were different, they were correlated with each reagent. The reference values inferred from the sVNT were Elecsys Anti-SARS-CoV-2: 71.8 U/L, ARCHITECT SARS-CoV-2 IgGII: 2976.3 AU/mL, Access SARS-CoV-2 IgG 1st IS: 689.6 IU/mL, and SARS-CoV-2 IgG S: 19.3 U/L.

Conclusions: The performance observed for each anti-SARS-CoV-2 antibody detection reagent was sufficient. The reference values based on the inhibition rate of sVNT have potential as indicators of the correlation of protection and are expected to be leveraged in automated antibody tests.

1. Introduction

In late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new coronavirus reported in Wuhan, posed a serious threat to global public health [1,2]. Currently, nucleic acid amplification tests and antigen tests are used for SARS-CoV-2 infection using nasopharyngeal swabs and saliva [3]; however, these tests may be affected by sample collection [4]. Alternatively, serological tests, which detect antibodies for pathogens in the blood, are expected to be useful for SARS-CoV-2 infection because they are less sensitive to sample collection and provide stable results [5,6]. In addition, since SARS-CoV-2 vaccination has been started worldwide, serological tests may be performed to confirm antibody production and to estimate the efficacy of the vaccine in vaccinated individuals [7,8]. To date, a variety of anti-SARS-CoV-2 antibody assay reagents have been developed and marketed as automated analyzers that are easy to use and have high sample throughput; however, their performance has not been sufficiently validated. Additionally, because the measurement value in quantitative reagents has not yet been standardized, the relationship between the measurement values in each reagent is not clear, and there

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Table 1
Characteristics of each reagent for anti-SARS-CoV-2 antibody.

Qualitative reagent	Instrument	Detection method	Immunoglobulin class	Antibody target	Cut-off value
Elecsys Anti-SARS-CoV-2	cobas e801	ECLIA	IgG+IgM	nucleocapsid	1.00 C.O.I.
ARCHITECT SARS-CoV-2 IgG	ARCHITECT i2000 SR	CLIA	IgG	nucleocapsid	1.40 S/C
ARCHITECT SARS-CoV-2 IgM	ARCHITECT i2000 SR	CLIA	IgM	spike RBD	1.00 S/C
Access SARS-CoV-2 IgM	Access 2	CLEIA	IgM	spike RBD	1.00 S/CO
SARS-CoV-2 IgM	Lumipulse L2400	CLEIA	IgM	spike RBD	1.00 C.O.I.
Quantitative assay reagent	**Instrument**	**Detection method**	**Immunoglobulin class**	**Antibody target**	**Cut-off value**
Elecsys Anti-SARS-CoV-2 S	cobas e801	ECLIA	IgG+IgM	spike RBD	0.80 U/mL
ARCHITECT SARS-CoV-2 IgG II	ARCHITECT i2000 SR	CLIA	IgG	spike RBD	50 AU/mL
Access SARS-CoV-2 IgG 1st IS	Access 2	CLEIA	IgG	spike RBD	30 IU/mL
SARS-CoV-2 S-IgG	Lumipulse L2400	CLEIA	IgG	spike RBD	1.0 AU/mL

Table 2
Repeatability and intermediate precision.

			Repeatability (n = 20)			Intermediate precision (n = 40)		
			Mean	SD	CV (%)	Mean	SD	CV (%)
Qualitative reagent	Elecsys Anti-SARS-CoV-2 (C.O.I.)	low	0.14	0.002	1.54	0.14	0.005	3.52
		medium	1.12	0.006	0.51	1.14	0.05	4.04
		high	6.42	0.04	0.59	6.82	0.27	3.91
	ARCHITECT SARS-CoV-2 IgG (S/C)	low	0.09	0.00	N.A.	0.10	0.004	4.90
		medium	0.75	0.01	1.85	0.77	0.01	1.89
		high	2.62	0.04	1.70	2.69	0.04	1.38
	ARCHITECT SARS-CoV-2 IgM (S/C)	low	0.70	0.02	3.54	0.73	0.03	4.52
		medium	1.68	0.04	2.27	1.22	0.04	3.56
		high	4.73	0.11	2.27	4.79	0.13	2.90
	Access SARS-CoV-2 IgM (S/CO)	low	0.51	0.04	7.72	0.48	0.03	7.21
		medium	1.06	0.06	5.45	0.93	0.06	6.02
		high	3.77	0.11	3.00	3.39	0.17	5.00
	SARS-CoV-2 IgM (C.O.I)	low	0.32	0.04	12.50	0.30	0.0	N.A.
		medium	1.14	0.06	5.00	1.01	0.05	4.95
		high	2.40	0.10	4.01	2.24	0.10	4.51
Quantitative reagent	Elecsys Anti-SARS-CoV-2 (U/mL)	low	0.66	0.01	1.77	0.67	0.03	3.86
		medium	1.26	0.03	2.17	1.16	0.06	5.49
		high	116.95	1.53	1.31	108.55	4.71	4.33
	ARCHITECT SARS-CoV-2 IgG II (AU/mL)	low	13.28	1.16	8.74	17.69	3.24	18.36
		medium	79.96	3.59	4.49	83.64	3.97	4.75
		high	597.0	17.29	2.89	620.83	17.59	2.83
	Access SARS-CoV-2 IgG 1st IS (IU/mL)	low	11.76	0.51	4.36	11.89	0.70	5.87
		medium	48.40	1.59	3.29	48.46	1.96	4.04
		high	397.89	14.76	3.71	381.98	18.58	4.86
	SARS-CoV-2 S-IgG (AU/mL)	low	0.60	0.0	N.A.	0.58	0.05	8.03
		medium	1.31	0.05	4.11	1.28	0.09	6.79
		high	25.42	0.96	3.79	23.44	0.97	4.13

N.A.: not available.

is currently a lack of evidence to interpret the results of antibody tests.

SARS-CoV-2 requires interaction between the receptor-binding domain (RBD) of the spike protein and angiotensin-converting enzyme II (ACE2) to infect cells [9]. Antibodies that inhibit this interaction are regarded as neutralizing antibodies and have attracted attention for their role in protecting the body against the viral infection. Most automated quantitative reagents have been designed to measure these antibodies; however, it has been reported that the measurement values obtained using such reagents do not necessarily reflect the amount of neutralizing antibody [10–12]. The conventional virus neutralization test, the gold standard for the measurement of neutralizing antibody titers, considerably limitation that requires handling live SARS-CoV-2 in a facility with biosafety level 3, and it takes several days to obtain the result. Therefore, a surrogate virus neutralization test (sVNT) was developed that can estimate the amount of neutralizing antibodies by the inhibitory reaction of ACE2 receptor protein binding to RBD. sVNT has a high correlation with the conventional test, does not require live virus or cells, and provides results in a few hours [13]. In addition, the most of functional assays like VNT are performed based on the independently protocol by laboratories and they are not standardized [14,15]. Although the evaluation of antibody measurement reagents by sVNT has been reported recently, the common indices that would be able to predict neutralizing antibody retention status in the measured values of each reagent have not been evaluated sufficiently [12].

Therefore, in this study, we compared the performance of various anti-SARS-CoV-2 antibody assay reagents in automated analyzers and explored the comparison method of measured values among the quantitative reagents.

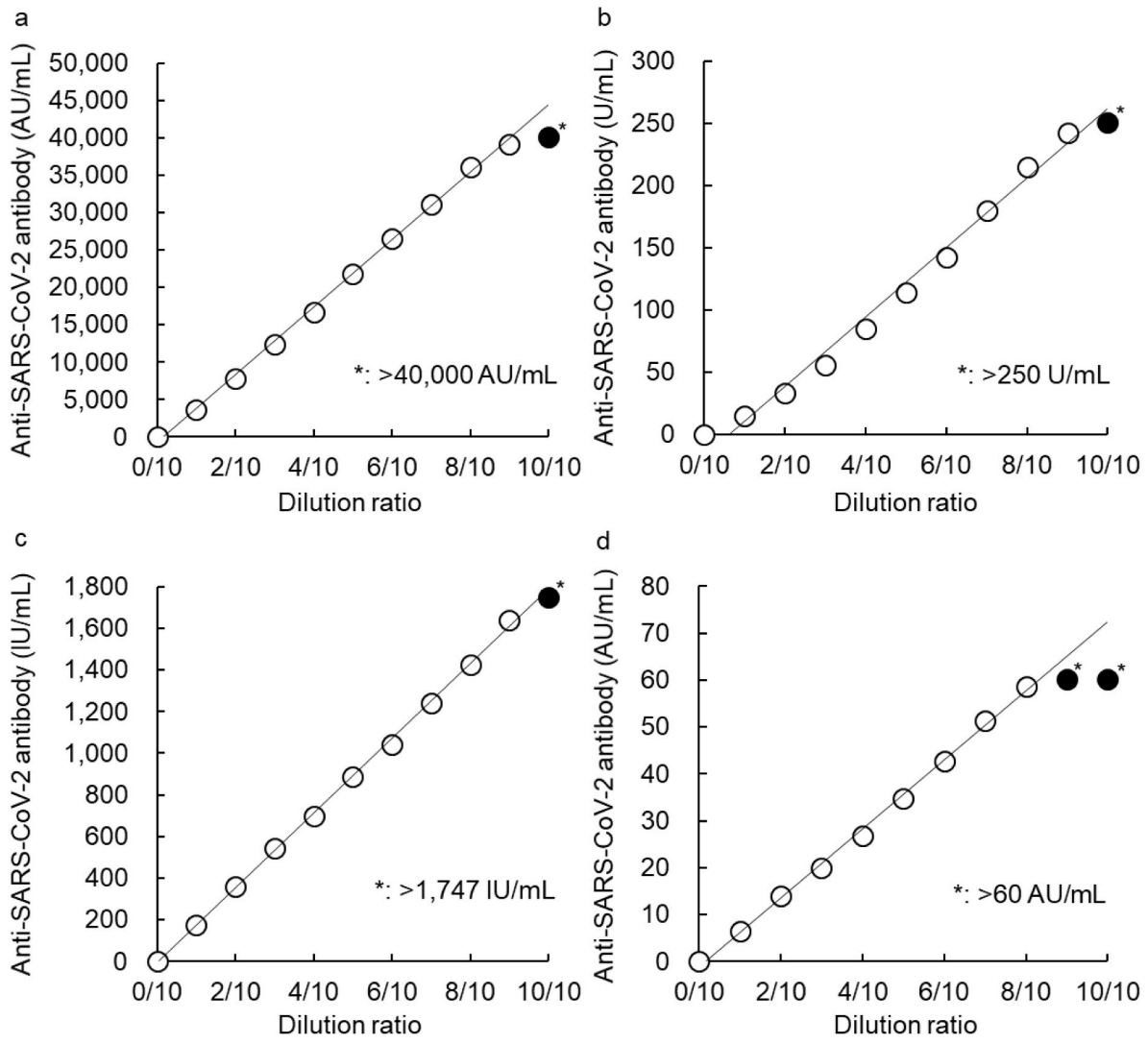

Fig. 1. Dilution linearity of quantitative reagent
Measurements of samples diluted in 10 steps with four quantitative reagents (a: ARCHITECT SARS-CoV-2 IgG II, b: Elecsys Anti-SARS-CoV-2, c: Access SARS-CoV-2 IgG 1st IS, and d: SARS-CoV-2 S-IgG). Open circles (○) indicate points in the range of linearity defined by the manufacturer. Closed circles with asterisks (●*) are values over the upper limit of measurement for each reagent. The solid line represents the regression line in the linearity range defined by the manufacturer.

2. Materials and methods

2.1. Sample collection

The present study was conducted on 100 serum samples collected from 100 unvaccinated patients diagnosed with COVID-19 between April 2020 and August 2021. All samples were aliquoted into 2 mL vials (Greiner Bio-One GmbH, Frickenhausen, Germany) and preserved at −80 °C until testing. All patients were confirmed for SARS-CoV-2 infection by nucleic acid tests or quantitative antigen tests [16,17].

2.2. Test for anti-SARS-CoV-2 antibody

Anti-SARS-CoV-2 antibodies were measured using five qualitative and four quantitative reagents (Table 1). Elecsys Anti-SARS-CoV-2 (Roche Diagnostics GmbH, Mannheim, Germany), ARCHITECT SARS-CoV-2 IgG (Abbott, Chicago, IL, United States), ARCHITECT SARS-CoV-2 IgM (Abbott, Chicago, IL, United States), Access SARS-CoV-2 IgM (Beckman Coulter, Brea, CA, United States), and SARS-CoV-2 IgM (Fujirebio Inc., Tokyo, Japan) were used as qualitative assay reagents. These IgG and IgM qualitative reagents detect antibodies against nucleocapsid and spike protein, respectively. The four quantitative reagents, detect antibodies against spike protein, were Elecsys Anti-SARS-CoV-2 S (Roche Diagnostics GmbH, Mannheim, Germany), ARCHITECT SARS-CoV-2 IgG II (Abbott, Chicago, IL, United States), Access SARS-CoV-2 IgG 1st IS (Beckman Coulter, Brea, CA, United States), and SARS-COV-2 IgG S (Fujirebio Inc., Tokyo, Japan). The reagents were loaded in their specialized instruments: cobas e801 (Roche Diagnostics GmbH, Mannheim, Germany), ARCHITECT i2000SR (Abbott, Chicago, IL, United States), Access 2 (Beckman Coulter, Brea, CA, United States), and Lumipulse L2400 (Fujirebio Inc., Tokyo, Japan). All assays were performed in accordance with the manufacturer's instructions.

2.3. Repeatability and intermediate precision

SARS-CoV-2 antibody-pooled serum was prepared at three levels (low: below the cut-off value; medium: around the cut-off value; high: levels over twice the cut-off value). The coefficient of variation (CV) of reproducibility was determined by consecutively measuring the pooled serum 20 times. To evaluate the concurrent accuracy, similar samples stored at −80 °C were measured twice a day for consecutive 20 days.

Table 3
Concordance between qualitative assay reagents

a

		Elecsys Anti-SARS-CoV-2		Total
		(+)	(−)	
ARCHITECT SARS-CoV-2 IgG	(+)	85	1	86
	(−)	5	9	14
Total		90	10	100
Kappa (95% CI): 0.72 (0.50-0.92)				

b

		Access SARS-CoV-2 IgM		Total
		(+)	(−)	
ARCHITECT SARS-CoV-2 IgM	(+)	89	1	90
	(−)	0	10	10
Total		89	11	100
Kappa (95% CI): 0.95 (0.84-1.00)				

c

		Access SARS-CoV-2 IgM		Total
		(+)	(−)	
SARS-CoV-2 IgM ARCHITECT	(+)	89	1	90
	(−)	0	10	10
Total		90	10	100
Kappa (95% CI): 0.95 (0.84-1.00)				

d

		SARS-CoV-2 IgM		Total
		(+)	(−)	
ARCHITECT SARS-CoV-2 IgM	(+)	90	0	89
	(−)	0	10	11
Total		90	10	100
Kappa (95% CI): 1.00 (1.00-1.00)				

Table 4
Results of two IgG antibody qualitative test reagents according to the days after onset in discrepant cases.

Discrepant case No.	Days after onset	ARCHITECT SARS-CoV-2 IgG ((+): >1.40 S/C)		Elecsys Anti-SARS-CoV-2 ((+): >1.00 C.O.I.)	
1	12	1.15	(−)	4.52	(+)
	13	2.60	(+)	19.9	(+)
2	14	0.09	(−)	2.25	(+)
	18	8.71	(+)	3.40	(+)
3	14	0.12	(−)	4.61	(+)
	22	1.45	(+)	4.13	(+)
4	18	2.81	(+)	0.769	(−)
	21	4.45	(+)	4.98	(+)
5	28	1.13	(−)	2.00	(+)
	30	4.39	(+)	82.2	(+)
6	31	1.31	(−)	2.89	(+)
	37	3.37	(+)	39.4	(+)

Table 5
Results of three IgM antibody qualitative test reagents according to the days after onset in discrepant cases.

Discrepant case No.	Days after onset	ARCHITECT SARS-CoV-2 IgM ((+): >1.00 S/C)		Access SARS-CoV-2 IgM ((+): >1.00 S/CO)		SARS-CoV-2 IgM ((+): >1.00 C.O.I.)	
7	24	2.59	(+)	0.61	(−)	2.3	(+)
	28	2.74	(+)	0.52	(−)	2.1	(+)

2.4. Dilution linearity

The samples containing high levels of anti-SARS-CoV-2 antibody beyond the upper limit of measurement in each quantitative reagent were diluted in 10 steps, and the linearity was evaluated from the average of three measurement values of each diluted sample.

2.5. Concordance rate of qualitative reagent and correlation of quantitative reagent

For the 100 serum samples collected, the concordance rate of judgment between Elecsys Anti-SARS-CoV-2 and ARCHITECT SARS-CoV-2 IgG and that for each of the three SARS-CoV-2 IgM antibody measurement reagents was examined. Furthermore, the kappa (κ) coefficient was used to assess the degree of agreement among the qualitative anti-SARS-CoV-2 antibody detection reagents. Similarly, the measurement values of the four quantitative reagents were compared using a regression equation and the correlation coefficient using the same samples. When the measurement values exceeded the upper limit of the measurable range of each reagent, the sample was diluted by each dedicated dilution solution to the range with dilution linearity, and the measurement values after dilution were multiplied by the dilution factor. We also compared the values obtained by converting each measurement value into bound antibody units per milliliter (BAU/mL) using the conversion factors related to the World Health Organization (WHO) International Standard for anti-SARS-CoV-2 immunoglobulin (human) (NIBSC Code 20–136) provided by each manufacturer to evaluate the degree to which the measurement values between each quantitative reagent were approximated by the correction factors. The manufacturer's conversion factors were as follows: Elecsys Anti-SARS-CoV-2 S, 0.972; ARCHITECT SARS-CoV-2 IgG II, 0.142; Access SARS-CoV-2 IgG 1st IS, 1.0; and SARS-CoV-2 IgG S, 12.0.

2.6. Neutralizing antibody detection and comparing measurement value of quantitative reagent

The neutralizing capacity against SARS-CoV-2 was evaluated using the SARS-CoV-2 surrogate virus neutralization test (Genscript, Piscataway, NJ, United States). The average of duplicate measurements was used as the measurement value for each sample, and all assays were performed according to the manufacturer's protocol. The inhibition rate by sVNT was plotted on the vertical axis, the measurement values of each quantitative reagent were plotted on the horizontal axis, and the logarithmic regression equation of each plot was calculated. Furthermore, using the results of a previous study as a reference, we estimated the measured value of each quantitative reagent corresponding to 84% inhibition, the median inhibition rate of sVNT in patients in the late stage of infection calculated by using a logarithmic regression equation [13].

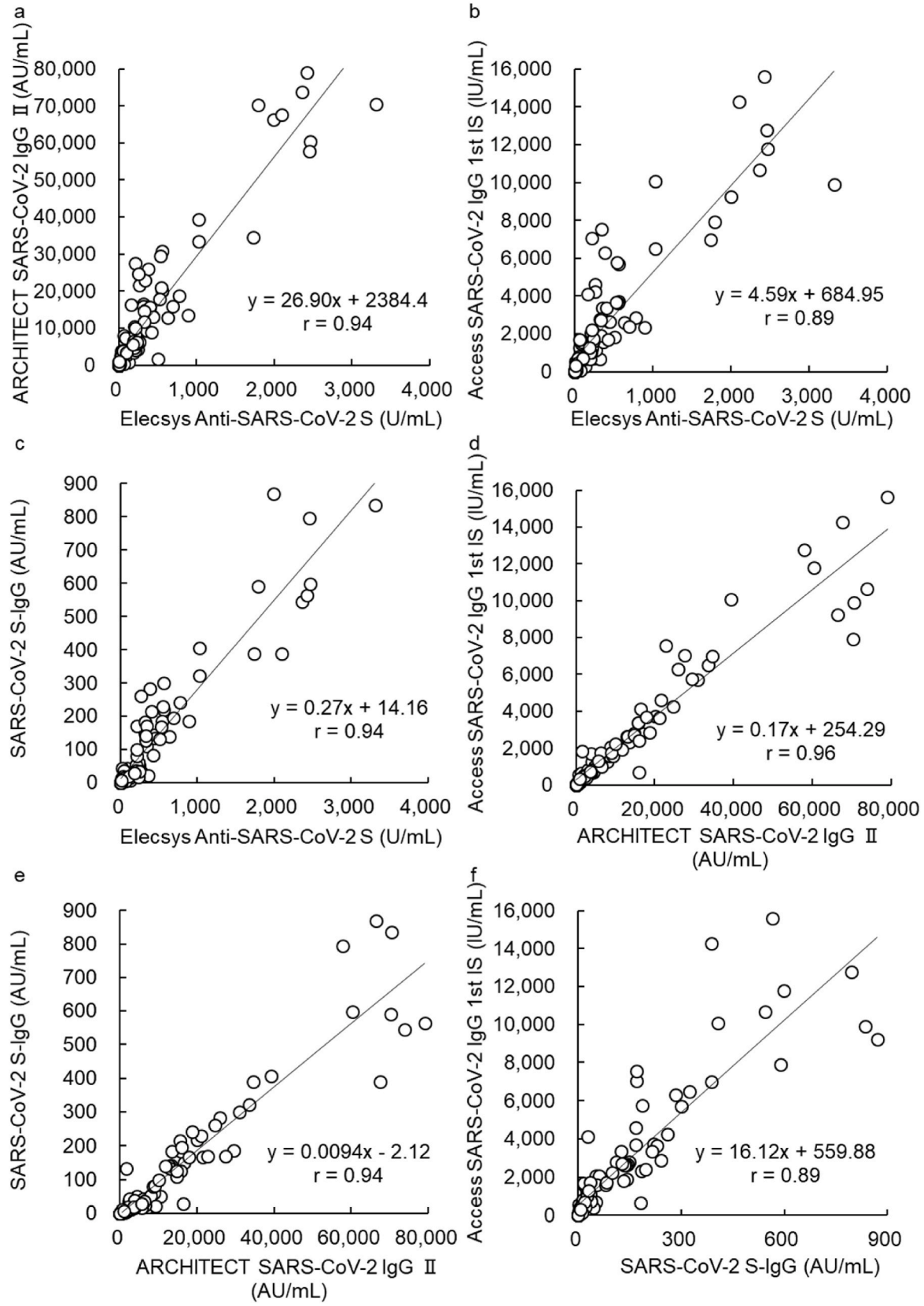

Fig. 2. Correlation in qualitative reagents for anti-SARS-CoV-2 antibody

Correlation between each quantitative reagent in 100 samples. a: correlation between ARCHITECT SARS-CoV-2 IgG II and Elecsys Anti-SARS-CoV-2 S, b: correlation between Access SARS-CoV-2 IgG 1st IS and Elecsys Anti-SARS-CoV-2 S, c: correlation between SARS-CoV-2 S-IgG and Elecsys Anti-SARS-CoV-2 S, d: correlation between Access SARS-CoV-2 IgG 1st IS and ARCHITECT SARS-CoV-2 IgG II, e: correlation between SARS-CoV-2 S-IgG and ARCHITECT SARS-CoV-2 IgG II, f: correlation between Access SARS-CoV-2 IgG 1st IS and SARS-CoV-2 S-IgG.

Table 6
Regression equation of measurement value of the quantitative reagent applied to each manufacturer's correction coefficient based on WHO standard products.

Vertical axis	Horizontal axis	Regression equation after adjustment to BAU/mL	Correlation coefficient	95% CI
ARCHITECT SARS-CoV-2 IgG II	Elecsys Anti-SARS-CoV-2 S	y = 3.93x + 338.59	0.94	0.90–0.96
Access SARS-CoV-2 IgG 1st IS	Elecsys Anti-SARS-CoV-2 S	y = 4.72x + 684.95	0.89	0.84–0.92
SARS-CoV-2 IgG S	Elecsys Anti-SARS-CoV-2 S	y = 3.31x + 169.89	0.94	0.91–0.96
Access SARS-CoV-2 IgG 1st IS	ARCHITECT SARS-CoV-2 IgG II	y = 1.21x + 254.29	0.96	0.93–0.97
SARS-CoV-2 IgG S	ARCHITECT SARS-CoV-2 IgG II	y = 0.79x - 25.46	0.94	0.92–0.96
Access SARS-CoV-2 IgG 1st IS	SARS-CoV-2 IgG S	y = 1.34x + 559.88	0.89	0.84–0.92

2.7. Statistical analysis

Statistical analyses, such as coefficient of variation, correlation analysis, and κ coefficient of concordance, were performed using the SAS Platform JMP Pro version 15.1.0. software (SAS Institute Inc., Cary, NC, United States).

2.8. Ethical approval

This study involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Institutional Review Board of Sapporo Medical University Hospital. https://web.sapmed.ac.jp/byoin/chiken/index.html (reference number 322–144).

2.9. Informed consent

Informed consent was obtained in the form of opt-out on the web-site (https://web.sapmed.ac.jp/la/200709_fujiya.pdf).

3. Results

The 100 serum samples were collected from 100 individual patients with COVID-19 with a median age of 63 years (range: 22–90 years), and 67 of them were male. According to the patients' electronic medical records, the median number of sample-collection days was 17 days (range: 1–39 days) after symptom onset.

3.1. Precision and linearity

The maximum CV of repeatability for qualitative and quantitative reagents was 12.50% (SARS-CoV-2 IgM: low-level) and 8.74% (ARCHITECT SARS-CoV-2 IgG II: low-level), respectively (Table 2). The intermediate precision ranged from 1.38 to 7.21% for qualitative reagents and 1.77–18.36% for quantitative reagents. In particular, ARCHITECT SARS-CoV-2 IgG II varied widely at levels below the cut-off value (SD: 3.24, CV: 18.36%). The repeatability of ARCHITECT SARS-CoV-2 and SARS-CoV-2 IgG S, and the intermediate precision of SARS-CoV-2 IgM could not be calculated because there was no variance in the measurement values of multiple measurement. The dilution linearity of each quantitative reagent was checked, and satisfactory linearity up to the upper limit of measurement defined by manufacturer was confirmed for all reagents (Elecsys Anti-SARS-CoV-2 S: 250.0 U/L, ARCHITECT SARS-CoV-2 IgG II: 40000.0 AU/mL, Access SARS-CoV-2 1st IS: 1747.0 IU/mL, SARS-CoV-2 IgG S: 60 AU/mL) (Fig. 1).

3.2. Agreement in the judgment of qualitative reagent

The judgment of two qualitative reagents that detected antibodies to nucleocapsids in the 100 serum samples was compared. As a result, 85 cases were positive and 9 cases were negative for both Elecsys Anti-SARS-CoV-2 and ARCHITECT SARS-CoV-2 IgG, and the concordance rate was 94%, and κ coefficient was 0.72 (95% IC: 0.50–0.92) (Table 3a). There were six discrepant samples between the two qualitative reagents of the IgG antibody. Similarly, comparing the determinations of the three reagents detecting IgM to spike proteins, there was one discrepant sample that was negative only for Access SARS-CoV-2 IgM (Table 3b–d). To investigate the causes of this discrepancy, in cases with discrepant judgement, the samples collected one-eight days later were measured. Among the qualitative reagents of IgG antibody for nucleocapsid, all negative judgments in six cases changed to positive in the re-evaluation (Table 4). On the other hand, in the reagent for measuring SARS-CoV-2 IgM, the result of Access SARS-CoV-2 IgM did not turn positive, even in the sample collected 4 days later (Table 5).

3.3. Correlation of the measurement values among quantitative reagents including adjustment to the common unit

The correlation between the four quantitative reagents was analyzed using the same sample. Although the measurement values of each reagent were different from the other, the correlation coefficient was 0.89–0.96, indicating a satisfactory positive correlation (Fig. 2a–f). The measurements were then converted to the common unit BAU/mL using the respective conversion factors provided by the companies and the regression equation was calculated in the same way (Table 6). The slopes of the regression equations were closer to 1.0 than before conversion in most combinations, although they still differed within the range of 0.79–4.72. In particular, the slopes of the regression equations with Elecsys Anti-SARS-CoV-2 S as the horizontal axis were large (3.31–4.72). However, there was no change in the correlation coefficients.

3.4. Comparison of measurement value of quantitative reagent with the inhibition rate of sVNT

To evaluate the measurement value at which the status of antibody production can be assumed to be produced in each reagent by the same indicator, each measurement value was compared with the inhibition rate of sVNT. The measurement value of each quantitative reagent was compared with the inhibition rate of sVNT using 49 samples, excluding 51 samples in which the measurement values of any quantitative reagent were below the cutoff value or over the upper limit of the measurement range. The measurement values corresponding to 84% of inhibition rate calculated from the logarithmic regression equation were Elecsys Anti-SARS-CoV-2: 71.8 U/L, ARCHITECT SARS-CoV-2 IgGII: 2976.3 AU/mL, Access SARS-CoV-2 IgG 1st IS: 689.6 IU/mL, and SARS-CoV-2 IgG S: 19.3 U/L (Fig. 3a–d).

4. Discussion

In the present study, we evaluated the performance and measurement values of various assay reagents for anti-SARS-CoV-2 antibodies using automated analyzers. The fundamental performance of all the reagents was sufficient. Although standardization of the measurement values of quantitative reagents still remains a problem, each reference value with a common indicator was considered useful.

The accuracy of all reagents was adequate for use in the clinical laboratory, and the quantification of measurement within each

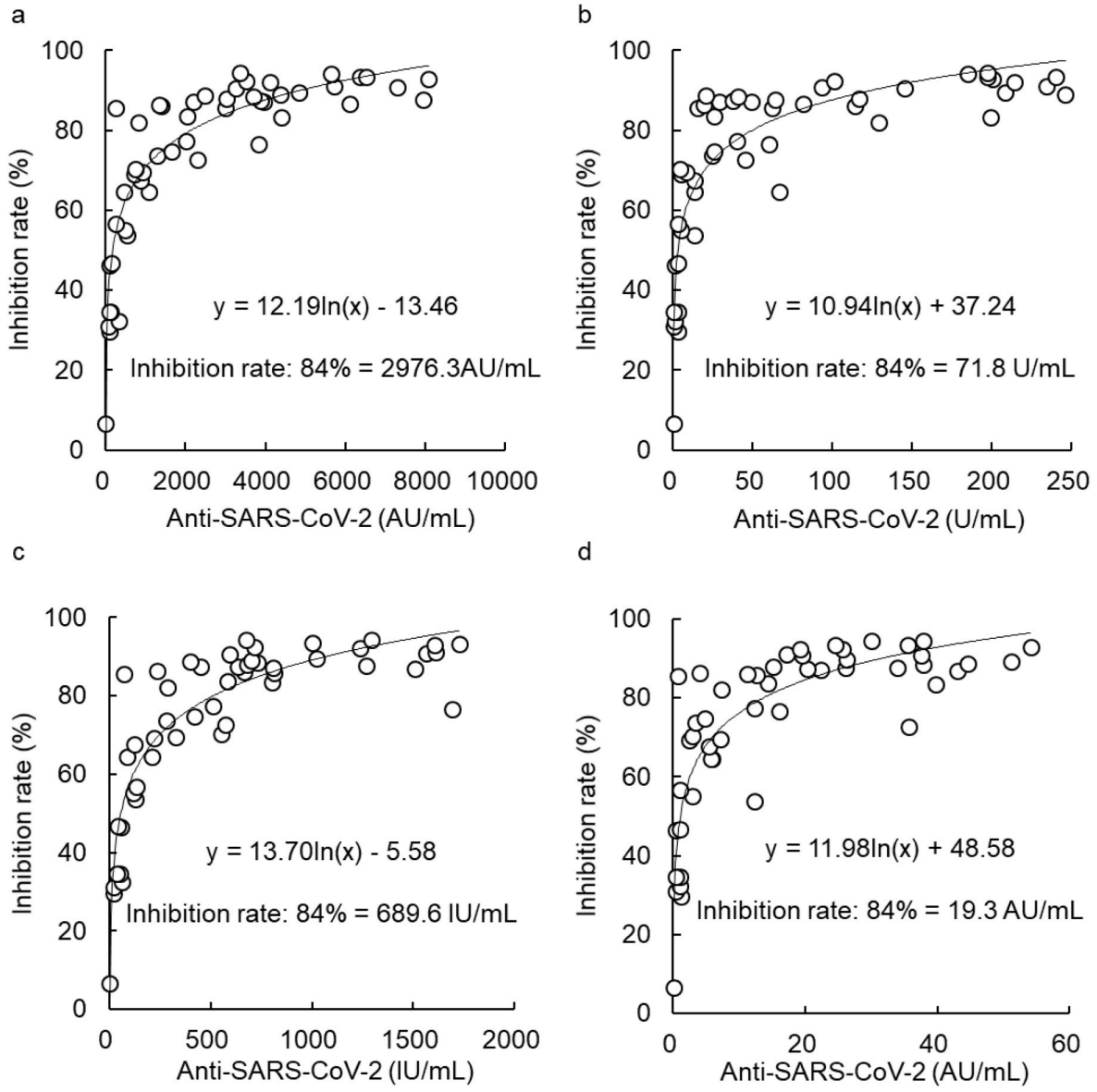

Fig. 3. Comparison of the inhibition rate by sVNT and the measurement values in various antibody quantitative reagents
Correlation between measurement values of each quantitative reagent and inhibition rate of sVNT in 49 positive samples (a: ARCHITECT SARS-CoV-2 IgG II, b: Elecsys Anti-SARS-CoV-2 S, c: Access SARS-CoV-2 IgG 1st IS, d: SARS-CoV-2 S-IgG). The solid line represents the approximate curve of the logarithmic regression.

measurement range was verified using four quantitative reagents. ARCHITECT SARS-CoV-2 IgG II, which had the highest CV with a maximum CV of 18.36% in intermediate precision, showed large variability at a level below the cutoff value. However, it was not considered a problem in actual laboratory tests because it was extremely unlikely to be involved in the positive or negative judgment considering the standard deviation, and the CV in the medium level, which was closer to the cutoff value, was also 4.75%, which is sufficiently acceptable.

On comparison of the two qualitative reagents for detecting antibodies against the nucleocapsid, six discrepant cases were observed. Because the results of samples collected afterwards turned positive, it suggested that Elecsys Anti-SARS-CoV-2 might be more sensitive and capable of early detection than ARCHITECT SARS-CoV-2 IgG. On the other hand, in the three qualitative reagents for SARS-CoV-2 IgM antibody detection, there was only one discrepant sample in which only Access SARS-CoV-2 IgM was negative. This sample was collected 24 days after onset, and the same result was obtained from the sample collected 4 days later, suggesting that the discrepancy could be caused by the reactivity of the reagent and not by time to antibody production.

Quantitative reagents indicate the amount of antibodies in the blood. Currently, various quantitative antibody reagents for SARS-CoV-2 have been developed; however, the units of measurement and cutoff values are different because of the different standards used. In fact, as shown in previous studies, the measurement values of the same sample using each reagent did not agree with each other in our study [12]. However, any combination of these four reagents yielded high correlation coefficients. The problem with practical use could be that it is difficult to compare and evaluate the measurement values of different reagents. After the launch of various antibody tests, the WHO defined an international standard for anti-SARS-CoV-2 antibodies [18]. Accordingly, each reagent manufacturer configured a correction formula that set the measurement value of each reagent to the value (BAU/mL) based on this international standard. Therefore, after applying each correction formula for the reagents to the measurement values of the quantitative reagents in this study, the correlation among the reagents was verified again. As a result, in most combinations, the slopes of the regression equations approached 1.0; however, the range of the slope was still 0.79–4.59, and the intercepts were still large. These results revealed that

it was difficult to uniformly compare the measurement values between each quantitative reagent even after correction. In particular, in Elecsys Anti-SARS-CoV-2 S, the slope of the regression equation after correction was in the range of 3.31–4.72. The corrected measurement values of Elecsys Anti-SARS-CoV-2 S tended to be lower than those of the other reagents, suggesting that more consideration is needed in the correction equation for this reagent. Standardization or harmonization is required so that measurement values can be compared and evaluated among reagents in the future.

Accordingly, we evaluated each measured value by comparing the inhibition rates obtained by sVNT. Tan et al. reported a median inhibition rate of 84% for sVNT in infected patients 14–61 days after disease onset [13]. In other words, this inhibition rate corresponds to neutralizing antibodies produced during the so-called late infection or recovery period by the immune response after SARS-CoV-2 infection [19,20]. Therefore, the values of each quantitative reagent corresponding to an inhibition rate of 84% in this sVNT kit could represent the value of neutralizing antibodies produced by patients in the late stage of infection. As shown in this study, the use of the reference value based on the inhibition rate of sVNT has the potential to evaluate the measurement value with a common standard. In addition, these reference values may serve as one of the indices of the correlates of protection because there is currently no index of infection protection capacity. Because it has been clarified that the amount of antibody chronologically decreases after vaccination, it is expected that multiple vaccinations will continue to be promoted [21,22]. Furthermore, the need for anti-SARS-CoV-2 antibody tests is predicted to increase in the future, as the timing of additional vaccinations and antibody titers after vaccination might be evaluated and determined by antibody test results. However, sVNT, which is currently used as a method for measuring neutralizing antibodies, is not suitable for processing a number of samples as a routine test because the measurement principle is based on enzyme-linked immunosorbent assays. From these viewpoints, anti-SARS-CoV-2 antibody tests using fully automated analyzers that are capable of handling a large number of samples and are easy to test can be useful as routine tests, and the results of this study may be helpful.

A limitation of this study is that the evaluation using negative samples was not conducted. Therefore, the frequency of false positives and distribution of measurement values in each reagent were not mentioned, and the cut-off value of each reagent needs to be reconsidered, as previously reported [23,24]. Vaccinated individuals were excluded from this study. The measurement value of antibodies and the inhibition rate of sVNT in vaccinated individuals may differ from that in infected individuals.

We found that each anti-SARS-CoV-2 antibody detection reagent for an automated analyzer has sufficient fundamental performance as a clinical examination method. To measure the value of quantitative reagents, we have shown a new index that corresponds to the amount of neutralizing antibodies in patients in the late stage of infection based on sVNT, which may be used as a reference for additional vaccination. In the future, it is hoped that antibody tests using such automatic analyzers will become more widely used and consequently contribute to the prevention of the infection.

5. Authorship statement

All authors meet the ICMJE authorship criteria. Contributors R.K., Y. F., and S.T. were responsible for the organization and coordination of the trial. S.T. was the chief investigator responsible for the data analysis. R.K., E.S., R.M., M.T., developed the trial design and conducted an investigation. All authors contributed to the writing of the final manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Declaration of competing interest

The authors declare the following conflict of interests which may be considered as potential competing interests: Satoshi Takahashi received speaker honoraria from MSD K.K. and research grants from Shino-Test Corporation, Roche Diagnostic K. K., Fujirebio Inc., and Abbott Japan Co., Ltd. All other authors declare no conflict of interests.

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