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Original Article

Factors affecting the sensitivity of quantitative severe acute respiratory syndrome coronavirus 2 antigen test

Yuki Sato [a,b], Ryosei Murai [a], Ryo Kobayashi [a,b], Atsuo Togashi [c], Yoshihiro Fujiya [b], Koji Kuronuma [d], Satoshi Takahashi [a,b,*]

[a] Division of Laboratory Medicine, Sapporo Medical University Hospital, Japan
[b] Department of Infection Control and Laboratory Medicine, Sapporo Medical University School of Medicine, Japan
[c] Department of Pediatrics, School of Medicine, Sapporo Medical University School of Medicine, Japan
[d] Department of Respiratory Medicine and Allergology, Sapporo Medical University School of Medicine, Japan

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ABSTRACT

Introduction: The accuracy of nucleic acid amplification tests (NAATs) is affected by various factors; however, studies examining the factors affecting the accuracy of quantitative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen test (QAT) are limited.

Methods: A total of 347 nasopharyngeal samples were collected from patients with coronavirus disease 2019 (COVID-19), and the date of onset was obtained from the electronic medical records. The SARS-CoV-2 antigen level was measured using Lumipulse Presto SARS-CoV-2 Ag (Presto), while NAAT was performed using the Ampdirect 2019-nCoV Detection Kit.

Results: Presto had a sensitivity rate of 95.1% (95% confidence interval: 92.8–97.4) in detecting the SARS-CoV-2 antigen in 347 samples. The number of days from symptom onset to sample collection was negatively correlated with the amount of antigen ($r = -0.515$) and sensitivity of Presto ($r = -0.711$). The patients' age was lower in the Presto-negative samples (median age, 39 years) compared with that in the Presto-positive samples (median age, 53 years; $p < 0.01$). A significant positive correlation was observed between age (excluding teenagers) and Presto sensitivity ($r = 0.764$). Meanwhile, no association was found between the mutant strain, sex, and Presto results.

Conclusion: Presto is useful for the accurate diagnosis of COVID-19 owing to its high sensitivity when the number of days from symptom onset to sample collection is within 12 days. Furthermore, age may affect the results of Presto, and this tool has a relatively low sensitivity in younger patients.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the coronavirus disease 2019 (COVID-19), which is mainly characterized by fever and respiratory symptoms [1]. SARS-CoV-2 is highly contagious [2]; therefore, SARS-CoV-2 testing plays an important role in preventing the spread of infection through early detection of infected individuals. The diagnostic tests for SARS-CoV-2 are categorized as follows: nucleic acid amplification tests (NAATs), antigen tests, and antibody tests. Antibody testing is employed for monitoring the prevalence of COVID-19, is not a substitute for virological testing, and should not be used for the diagnosis of acute SARS-CoV-2 infection [3]. In January 2023, NAATs and antigen tests were the primary methods used to confirm SARS-CoV-2 infections in Japan. A positive result in either test confirmed the presence of infection at the time of sample collection. However, samples with a low viral load may test negative for SARS-CoV-2, or the viral load might change over time, even in the same patient; thus, samples must be collected at the appropriate time. The quantitative SARS-CoV-2 antigen test (QAT) can be employed to quantitatively measure the antigen levels utilizing appropriate instruments

Abbreviations: CI, confidence interval; COVID-19, coronavirus disease 2019; IQR, interquartile range; NAAT, nucleic acid amplification test; QAT, quantitative severe acute respiratory syndrome coronavirus 2 antigen test; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

* Corresponding author. Department of Infection Control and Laboratory Medicine, Sapporo Medical University School of Medicine, South-1 West-16, Chuo-ku, Sapporo, 060-8543, Japan.
E-mail address: stakahas@sapmed.ac.jp (S. Takahashi).

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and reagents based on the principles of certain detection technologies such as chemiluminescent enzyme immunoassays. Furthermore, previous meta-analyses have reported the high sensitivity, specificity, and predictive value of QAT [4,5]. NAAT might show a positive result, while QAT could show a negative result when the interval from symptom onset to sample collection is long [6]; however, only a few studies reported the sensitivity rate of these tests each day. Therefore, we aimed to investigate whether the number of days from symptom onset to sample collection and other factors affected the QAT sensitivity.

2. Materials and methods

2.1. Sample collection

A total of 372 nasopharyngeal samples were collected from COVID-19 patients who were admitted in Sapporo Medical University Hospital between March 2021 and September 2021. The samples were collected by the physician in charge using UTM 305C swabs (Copan Diagnostics, Murrieta, CA, USA). QAT was performed immediately after collection, and NAATs and SARS-CoV-2 mutation analysis were kept for up to 48 h at 2–8 °C until analysis.

2.2. QAT

QAT was performed using Lumipulse Presto SARS-CoV-2 Ag (Fujirebio Inc., Tokyo, Japan) according to the manufacturer's instructions. Presto was analyzed using the fully automated Lumipulse® L2400 (Fujirebio Inc., Tokyo, Japan). The test results were deemed negative if the SARS-CoV-2 Ag level was less than 1.00 pg/mL and positive if it was more than or equal to 1.00 pg/mL [6]. If the upper limit of 10,000 pg/mL exceeded, a cut-off level of 10,000 pg/mL was set.

2.3. NAATs

NAATs were performed using the Ampdirect 2019-nCoV Detection Kit (Shimadzu Corporation, Kyoto, Japan; Ampdirect), according to the manufacturer's instructions. The N1 and N2 primer-probe sets of Ampdirect have the same nucleotide sequences as the primer-probe set recommended by the Centers for Disease Control and Prevention [7]. The detection limits of Ampdirect using artificially synthesized RNA had a sufficient "detection power" of 1000 copies/mL for N1 and 4200 copies/mL for N2 [8]. A mixture of the sample (5 μL) and treatment solution (5 μL) was pretreated at 90 °C for 5 min and then added to the PCR solution. Amplification and real-time detection were performed using a LightCycler 480 system (Roche, Basel, Switzerland). The test results were deemed positive when an increase in the amplification curve was observed during the reaction time in either or both the N1 and N2 primer sets.

2.4. SARS-CoV-2 mutation analysis

RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. RNA was extracted and purified from 140 μL of each sample, and finally eluted in 60 μL of elution buffer. Mutant strains of SARS CoV-2 were analyzed using LightCycler® 480 System and five reagents: VirSNiP SARS-CoV-2 Spike N501Y (Roche, Basel, Switzerland), VirSNiP SARS-CoV-2 Spike del H69/V70 (Roche, Basel, Switzerland), VirSNiP SARS-CoV-2 Spike D614G (Roche, Basel, Switzerland), VirSNiP SARS-CoV-2 Spike P681H (Roche, Basel, Switzerland), and VirSNiP SARS-CoV-2 Spike Y453F (Roche, Basel, Switzerland).

2.5. Assessment methods

Data of patients' age, sex, and date of symptom onset were retrospectively collected from the electronic medical records. The sensitivity of Presto was analyzed by stratifying the days from symptom onset to sample collection into the following categories using Ampdirect as a reference for comparison: 0 days, 1–3 days, 4–6 days, 7–9 days, and ≥10 days. We also evaluated the Presto sensitivity and SARS-CoV-2 antigen levels each day from the onset of symptoms to sample collection; the sensitivity of Presto was calculated in three or more samples each day. Statistical analysis was performed to determine the relationship between SARS-CoV-2 antigen levels, mutant strain, age, sex, and cycle threshold (Ct) values based on the Ampdirect and Presto' s judgment results.

2.6. Statistical analysis

Statistical analysis of quantitative variables was performed using the Shapiro-Wilk test to confirm normality; then, the results were evaluated using the Mann-Whitney *U* test, Welch's *t*-test, correlation coefficient, and test for correlation. Qualitative variables were analyzed using the χ^2 test. All statistical analyses were performed using IBM SPSS Statistics version 24 (IBM Corp., Chicago, IL, USA). A p value of <0.05 was considered significant.

2.7. Ethical statement

This study was approved by the Clinical Research Review Committee of Sapporo Medical University Hospital (approval number: 332–3204). The requirement for informed consent was waived owing to the retrospective nature of the study. An opportunity to indicate their intentions not to participate in this study was provided by opting out.

3. Results

3.1. Clinical overview

A total of 347 samples were collected from patients, whose symptom onset was determined, at a median time of 7 days (range, 0–24 days, Supplementary Table 1). There was no difference in the number of days from symptom onset to sample collection by age groups ($p = 0.50$, r = 0.032, Supplementary Fig. 1). The median age of patients with COVID-19 was 53 years (range, 14–88 years); of the total patients, 188 were men and 159 were women.

3.2. Sensitivity of QAT

We analyzed the sensitivity of Presto using Ampdirect as a control (Table 1). The sensitivity of Presto in 347 samples was 95.1% (95% confidence interval [CI]: 92.8–97.4). The sensitivity rates of Presto from the symptom onset to sample collection were as follows: 100.0% at 0 day and 1–3 days, 93.5% (95% CI: 88.6–98.5) at 4–6 days, and 96.4% (95% CI: 93.4–99.5) at 7–9 days; meanwhile, the sensitivity of Presto in samples collected after 10 days of symptom onset decreased to 90.0% (95% CI: 82.4–97.6).

3.3. Trends in sensitivity and antigen level of QAT from symptom onset to sample collection

The median SARS-CoV-2 antigen level tended to decrease over time (Supplementary Table 2). The number of days from symptom onset to sample collection was negatively correlated with SARS-CoV-2 antigen levels ($p < 0.01$, r = −0.515, Fig. 1) and sensitivity of Presto ($p < 0.01$, r = −0.711, Fig. 2). The sensitivity of Presto was also comparable to that of NAATs, which never decreased to below 90% within 12 days from symptom onset (Fig. 2).

3.4. Relationships between the results of QAT and response variable

The age of patients with Presto-negative samples (median age, 39; interquartile range [IQR], 31–45 years) was significantly lower than that

Table 1
Sensitivity of quantitative reagent used for detecting SARS-CoV-2 antigen by days from onset to sample collection.

		Ampdirect 2019-nCoV Detection Kit (+)					
		All	0 days	1–3 days	4–6 days	7–9 days	≥10 days
Lumipulse Presto SARS-CoV-2 Ag	(+)	330	3	51	87	135	54
	(−)	17	0	0	6	5	6
Total		347	3	51	93	140	60
Sensitivity (%), (95% CI)		95.1 (92.8–97.4)	100[a]	100[a]	93.5 (88.6–98.5)	96.4 (93.4–99.5)	90.0 (82.4–97.6)

CI, confidence interval; +, positive; −, negative.
[a] 95% CI could not be calculated.

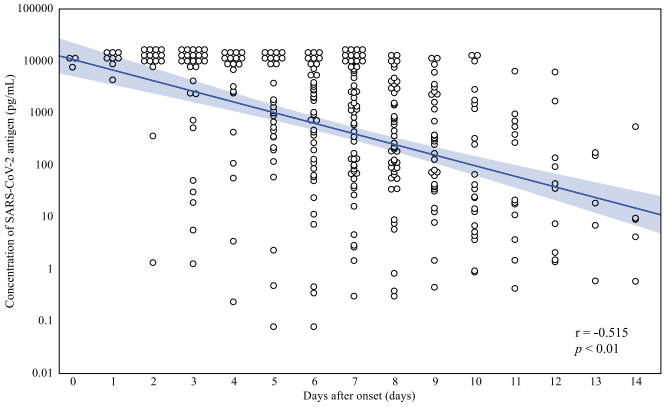

Fig. 1. Trends in the levels of SARS-CoV-2 antigen by number of days from symptom onset to sample collection. The blue slope line represents the linear regression. The gray shadow indicates the 95% confidence interval around the linear regression.
Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

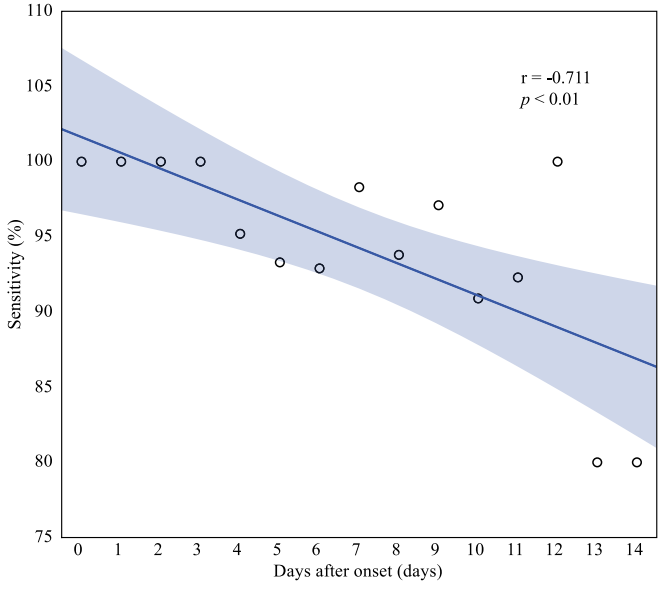

Fig. 2. Trends in the sensitivity of quantitative reagent used for detecting SARS-CoV-2 antigen by number of days from symptom onset to sample collection. The blue slope line represents the linear regression. The gray shadow indicates the 95% confidence interval around the linear regression. The sensitivity of each reagent was determined in three or more samples.
Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

of patients with Presto-positive samples (median age, 53 years; IQR, 42–66 years; $p < 0.01$; Fig. 3). No correlation was found between age and Presto sensitivity ($p = 0.30$, r = 0.415, Fig. 4A). However, a

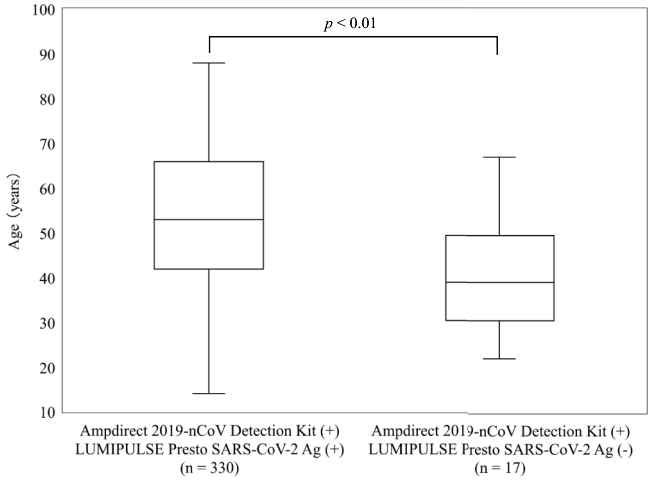

Fig. 3. Relationship between the result of quantitative reagent used for detecting SARS-CoV-2 antigen and age. The horizontal line in the middle of the box is the median value and the lower and upper boundaries denote the 25th and 75th percentiles, respectively. The largest and smallest values are also shown, with lines are drawn from the ends of the box to those values. The *p*-value was calculated using the Mann–Whitney *U* test. Abbreviations: (+), positive; (−), negative; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

significant positive correlation ($p = 0.04$, r = 0.764, Fig. 4B) was observed between these two factors when teenagers were excluded: seven teenagers showed positive Presto results.

For all samples, the Ct values obtained based on the Ampdirect results were significantly higher for Presto-negative samples than for Presto-positive samples ($p < 0.01$; Fig. 5). By contrast, no association was found between Presto sensitivity for samples collected within 9 days and that of samples collected after 10 days of symptom onset ($p = 0.09$, Table 2). Similarly, no association was observed between the mutant strain, sex, and Presto results (Table 2). Furthermore, no apparent association was found between the mutant strains and SARS-CoV-2 antigen levels (p=0.07; Fig. 6).

4. Discussion

In this study, we evaluated whether the number of days from symptom onset to sample collection and other factors affect the Presto sensitivity. As a result, the sensitivity rate of Presto was 98.3% (95% CI: 95.0–100.0), which is comparable to that reported by Fujiya et al. [9]. Singanayagam et al. reported that the probability of culturing SARS-CoV-2 after 10 days from symptom onset was 6% [10], which may have affected the SARS-CoV-2 antigen levels and Presto sensitivity. The Ct values obtained based on the Ampdirect results were significantly higher for Presto-negative samples than for Presto-positive samples. Therefore, Presto-negative samples contained trace amounts of SARS-CoV-2 RNA; moreover, the Ampdirect may have detected the

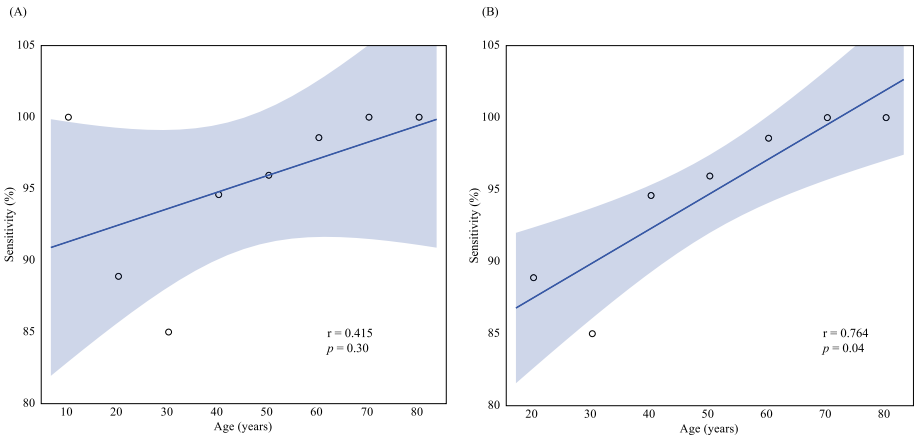

Fig. 4. Correlation between sensitivity of quantitative reagent used for detecting the SARS-CoV-2 antigen and age. The blue slope line represents the linear regression. The gray shadow indicates the 95% confidence interval around the linear regression. (A) All ages. (B) Excluding teenagers
Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

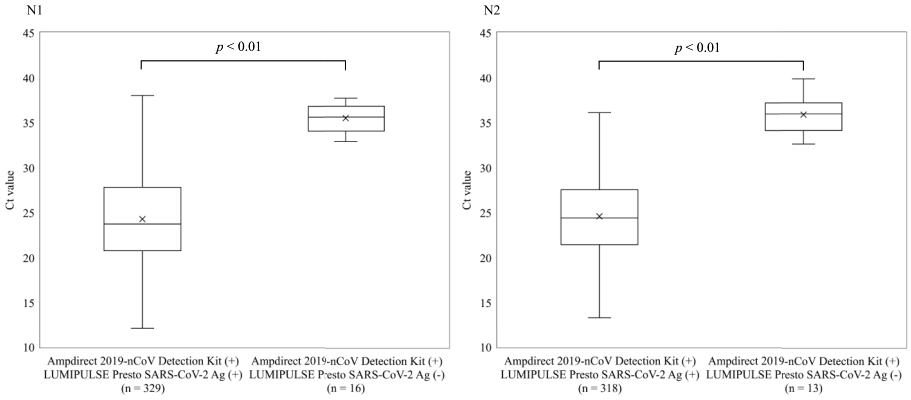

Fig. 5. Relationship between the result of quantitative reagent used for detecting SARS-CoV-2 antigen and the cycle threshold values of Ampdirect 2019-nCoV Detection Kit. The horizontal line in the middle of the box is the median value and the lower and upper boundaries denote the 25th and 75th percentiles, respectively. The largest and smallest values are also shown, with lines are drawn from the ends of the box to those values. The *p*-value was calculated by the *t*-test. Abbreviations: (+), positive; (−), negative; × , average; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ct, cycle threshold.

Table 2
Relationships between the results of the quantitative reagent used for detecting SARS-CoV-2 antigen (predictor variable) and response variables.

		LUMIPULSE Presto SARS-CoV-2 Ag		*p*-value
		(+), (%)	(−), (%)	
Days from onset to sample collection	≤9	276 (96.2)	11 (3.8)	0.09[a]
	>9	54 (90.0)	6 (10.0)	
SARS-CoV-2 variant	Alfa variants	213 (98.2)	4 (1.8)	0.99[a]
	Delta variants	90 (97.8)	2 (2.2)	
Sex	Man	177 (94.1)	11 (5.9)	0.37[a]
	Women	153 (96.2)	6 (3.8)	

+, positive; −, negative.
[a] χ^2Test.

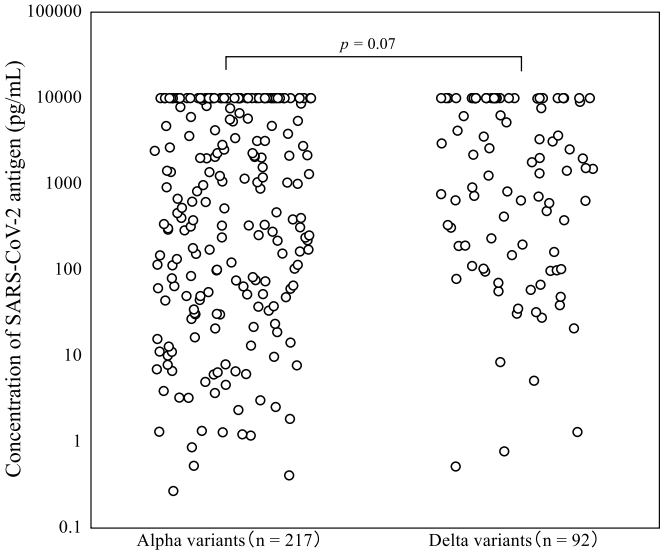

Fig. 6. Relationship between concentration of SARS-CoV-2 antigen and the variant pattern. The *p*-value was calculated using the Mann–Whitney *U* test.
Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

presence of non-infectious SARS-CoV-2 [11].

We investigated whether age and mutant strains affected the Presto results. The SARS-CoV-2 viral load in posterior oropharyngeal saliva samples is low in younger people and high in older people [12]. In addition, the SARS-CoV-2 viral load correlates with SARS-CoV-2 antigen levels [13]. Inevitably, the results of Presto would also correlate with age, as demonstrated in this study, which was assessed with nasopharyngeal samples. The increased SARS-CoV-2 viral load in older individuals may be attributed to the impaired efficacy of the innate and adaptive immune systems caused by macrophage dysfunction observed with aging [14], although this finding remains uncertain. On the

contrary, the evaluation of the correlation between age and the sensitivity of Presto showed no significant difference for all ages. Except in teenagers, the Presto sensitivity increased significantly with increasing age. Teenagers had a small number of samples, and all samples showed positive results on Presto staining, which may have affected the evaluation of all age groups. In this study, the alpha and delta variants were detected, but they did not affect the Presto results. Presto quantitatively measures the antigen levels of the nucleocapsid protein of SARS-CoV-2; the alpha and delta variants are characterized by mutations in spike proteins [15]; thus, the results of Presto were not affected. The omicron variants named by the World Health Organization in November 2021 also demonstrate mutations in spike proteins, and these variants will not have an effect on the Presto results. The delta variants had significantly higher SARS-CoV-2 viral load than the alpha variants in saliva [16]. In this study, no significant difference was observed between the results of mutant strains and SARS-CoV-2 antigen levels.

This study has two limitations, however. First, a possible limitation of this study is that the analysis was performed based on a SARS-CoV-2 antigen cut-off level of 10,000 pg/mL as the level exceeded 10,000 pg/mL. This may have influenced the significant difference between the results of the mutant strains and the antigen levels. Second, we did not perform culture of virus because of the time required to culture the cells and confirm viral. Therefore, we were unable to validate the sensitivity of Presto using SARS-CoV-2 cultures as a control.

In conclusion, Presto is useful for the accurate diagnosis of COVID-19 owing to its high sensitivity when the time from symptom onset to sample collection is less than 12 days. Furthermore, age may affect the results of Presto, which has a relatively low sensitivity in younger patients.

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Authorship statement

All the authors meet the ICMJE authorship criteria. YS, RM, AT, YF, KK, and ST contributed to the organization and coordination of the trial. ST was the chief investigator and was responsible for the data analysis. YS, RM, RK developed the trial design and conducted the investigation. All authors contributed to the writing of the final manuscript.

Declaration of competing interest

The authors declare the following potential competing interests: Satoshi Takahashi received speaker honoraria from MSD K.K. and Fujirebio Inc. and research grants from Shino-Test Corporation, Roche Diagnostic K.K., Fujirebio Inc., and Abbott Japan Co., Ltd. The rest of the authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2023.04.005.

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