

Methods and Applications

Development and Diagnosis Performance of IgM-Based Rapid Antigen Test for Early Detection of SARS-CoV-2 Infection in a Large Cohort of Suspected COVID-19 Cases — USA, Poland, and Sweden, 2021–2022

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ABSTRACT

Introduction: Antigen testing has been crucial in effectively managing the coronavirus disease 2019 (COVID-19) pandemic. This study evaluated the clinical performance of a nasopharyngeal swab (NPS)-based antigen rapid diagnostic test (Ag-RDT) compared to the gold standard real-time reverse transcription-polymerase chain reaction (RT-PCR) for early detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: We developed an IgM-based rapid antigen test for early detection of SARS-CoV-2 infection. Between July 2021 and January 2022, we analyzed 1,030 NPS samples from participants at three centers in different countries, using both antigen rapid diagnostic tests (Ag-RDT) and RT-PCR.

Results: The Ag-RDT demonstrated minimal detection limits as low as 0.1 ng/mL for recombinant N antigen and 100 TCID₅₀/mL for heat-inactivated SARS-CoV-2 virus. Specificity assessments involving four human coronaviruses and 13 other respiratory viruses showed no cross-reactivity. The Ag-RDT assay (ALLtest) exhibited high sensitivity (93.18%–100%) and specificity (99.67%–100%) across all centers. Factors such as cycle threshold (Ct) values and the timing of symptoms since onset were influential, with sensitivity increasing at lower Ct values (<30) and within the first week of symptoms.

Conclusion: The ALLtest Ag-RDT demonstrated high reliability and significant potential for diagnosing suspected COVID-19 cases.

syndrome coronavirus 2 (SARS-CoV-2), has imposed an unprecedented burden on public health systems worldwide. As of mid-July 2023, there have been over 768 million confirmed cases of SARS-CoV-2 infection, resulting in more than 6.9 million deaths globally (1). The rapid increase in new cases continues to be fueled by various genomic variants of the virus. Notably, the recent emergence of the BA.1 through BA.5 and XBB (Omicron) variants, classified as the sixth variant of concern, demonstrates increased transmissibility and resistance to immunity (2).

SARS-CoV-2 transmits among humans via respiratory droplets and aerosols during actions such as sneezing, talking, or coughing (3). The rapid and widespread dissemination of SARS-CoV-2 across geographical and species boundaries highlights the critical need for adopting the One Health approach to effectively tackle public health emergencies. Central to managing the COVID-19 pandemic is the development and implementation of swift and accessible diagnostic tools. Currently, real-time reverse transcription-polymerase chain reaction (RT-PCR) is considered the benchmark for COVID-19 diagnosis; however, challenges persist in its practical use. Notably, during the collection of nasopharyngeal secretions, the recommended one-meter distance between the healthcare provider and the patient is often not maintained, potentially heightening the risk of COVID-19 transmission. Furthermore, RT-PCR can take several hours to yield results and may result in false negatives (4). Consequently, developing a rapid detection method is imperative for timely isolation of suspected SARS-CoV-2 cases and minimizing the transmission risk.

The SARS-CoV-2 antigen rapid diagnostic test (Ag-RDT) has been crucial in the early detection of cases, containment strategies, and reducing transmission (5–7). Multiple studies have assessed Ag-RDTs'

Coronavirus disease 2019 (COVID-19), a contagious illness caused by the severe acute respiratory

diagnostic accuracy across different populations and clinical environments, revealing varied results (8–10). Ag-RDTs demonstrate high sensitivity during the initial week following symptom onset and exhibit excellent specificity when viral loads are elevated (5). Currently, Ag-RDTs are accessible to the general public, delivering results within 15–30 minutes. Consequently, they serve as a supplementary method to RT-PCR for the quick, economical, and straightforward identification of SARS-CoV-2 infections. Furthermore, Ag-RDTs are effective as point-of-care tests (POCT) for diagnosis in the disease's early stages, providing a significant advantage in curbing SARS-CoV-2 spread.

In the conceptual design phase, our ALLtest distinguishes itself from other Ag-RDTs through its innovative utilization of IgM antibodies targeting the nucleocapsid (N) protein. The N protein is integral to various stages of the replication cycle and serves as a serological marker for SARS-CoV-2 infection (11). This technique enhances early-stage infection detection and provides deeper insights into transmission dynamics. By incorporating this novel element into antigen diagnosis, the ALLtest enables a more comprehensive evaluation of SARS-CoV-2 infection, thereby facilitating improved public health interventions and mitigation strategies. In this study, nasopharyngeal swabs (NPS) were collected from suspected COVID-19 cases and subjected to diagnostic RT-PCR in the United States (US), Sweden (SE), and Poland (PL) from July 2021 to January 2022. All samples were subsequently analyzed using the ALLtest to conduct a detailed correlation analysis among various diagnostic assays. Furthermore, we explore the challenges and limitations of IgM-based antigen diagnosis, underscoring the necessity for versatile diagnostic approaches to effectively address the global health crisis. By elucidating the details of this innovative methodology, we not only enhance our comprehension of COVID-projected diagnostics but also contribute to establishing a more robust and proactive global health response.

METHODS

Preparation and Characterization of Monoclonal Antibodies (mAbs) Against SARS-CoV-2 N Protein

The nucleocapsid protein (NCBI: NC_045512.2) was expressed under identical conditions as described in our previous study (12). BALB/c mice, ranging in

age from 4 to 8 weeks, were intraperitoneally injected with 50 µg of N antigen per mouse for the initial immunization, followed by 100 µg per mouse for the second and third immunizations at biweekly intervals. Spleen cells from mice with high antibody titers were fused with myeloma cells to produce hybridoma cells. These cells were then injected into BALB/c mice to generate mAbs specific for the SARS-CoV-2 N protein. The titers of these mAbs were evaluated using indirect ELISA on plates coated with 5 µg/well of N antigen. We tested 12 distinct mAbs, initially diluted from 1:10 to 1:10⁸ starting from a concentration of 1 mg/mL. Pair screening of the mAbs was conducted using the ForteBio Octet[®] Red96 system, where the response value (nm) indicative of specific binding to the SARS-CoV-2 N antigen was recorded for each mAb pair. High-titer mAbs demonstrating effective pairing were further characterized for their isotypes using the multiple antibody isotypes kit from Proteintech Group Inc., China (Cat No. PK20003), which includes IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, kappa light chain, and lambda light chain.

The Design of Ag-RDT Assay for COVID-19

The SARS-CoV-2 Ag-RDT was developed using the immunochromatographic method to detect the SARS-CoV-2 N antigen in NPS samples. The structural design of the Ag-RDT cassette is principally similar to that used in the ALLtest for antibody detection (13). The process begins by placing the sample and buffer on the sample pad. The fluid then migrates from left to right, sequentially interacting with the conjugation pad that contains labeled colloidal gold-mAb17, the NC membrane where specific antibodies are immobilized at distinct lines [test (T) line with mAb16 at 1 mg/mL, and control (C) line with goat anti-mouse IgG at 1 mg/mL], and finally the absorption pad.

Determination of the ALLtest Ag-RDT Assay Sensitivity and Specificity

To assess the sensitivity of the ALLtest Ag-RDT Assay, preparations were made using 1.0×10⁶ median tissue culture infectious doses (TCID₅₀)/mL of the SARS-CoV-2 Shenzhen/02/2020 strain and 1 mg/mL of full-length nucleocapsid protein (NCBI: NC_045512.2). For specificity validation of the same assay, samples from four human coronaviruses (HCoV-229E, HCoV-HKU1, CoV-OC43, HCoV-NL63), parainfluenza viruses 2 and 3, influenza A and B,

adenovirus types 3 and 7, human rhinovirus 2, 14, and 16, measles virus, mumps virus, and respiratory syncytial virus were prepared. Furthermore, to evaluate potential interference, substances, including budesonide nasal spray, mucin, dexamethasone, flunisolide, mupirocin, oxymetazoline, phenylephrine, rebetol, reenza, tamiflu, tobramycin, and whole blood, were spiked with negative and weak positive SARS-CoV-2 samples. Swabs spiked with virus tissue cultures, nucleocapsid protein, various virus strains, and interfering substances at specified concentrations were tested under defined reaction conditions.

Evaluation of the Application of the SARS-CoV-2 Variant Strains

To assess the sensitivity of the ALLtest Ag-RDT Assay across various SARS-CoV-2 variants, including Alpha, Beta, VUI-21ARP-03, Gamma, Delta, and Omicron, these strains were prepared in dilutions using 0.5% BSA-PBS to create different concentration solutions. A volume of 30 μ L from each solution was applied to the tip of a nasal swab, subsequently immersed in extraction buffer. Three to four drops of this mixture were then placed into the sample well of the test cassette. Results were visually read from the cassette after 15 minutes, according to the protocol specified by the manufacturer.

Validation of Clinical Samples

Antigen tests were conducted on 1,030 individuals

suspected of having COVID-19 across three international centers: Phamatech, Inc. in America (PI-US), iLab in Sweden (I-SE), and Uniwersyteckie Centrum Kliniczne in Poland (UCK-PL) from July 2021 to January 2022. Participant data from these centers is compiled in Table 1. Each participant provided two NPS specimens. One swab was analyzed immediately using the ALLtest Ag-RDT Assay or processed using sample lysates without protein denaturant and stored at either 4 °C for up to 24 hours or below -70 °C for extended storage. The second swab was preserved in universal transport medium and tested for ORF1a/b through quantitative reverse transcription PCR (qRT-PCR) within 24 hours. The Ct values from the qRT-PCR were documented for subsequent analysis. This research received ethical approval from the Ethical Committee of Phamatech, Inc. in America (Approval No. 21-HANG-101) and Uniwersyteckie Centrum Kliniczne in Poland (Approval No. NKBBN/710/2021). The requirement for informed consent was waived for the clinical trial conducted by iLab in Sweden.

Statistical Analysis

The sensitivity and specificity served as the primary metrics for assessing our antigen test. Sensitivity, or the true positive rate, was determined by dividing the number of positive cases identified by the ALLtest by the number of true positive cases confirmed by the RNA test. Specificity, or the true negative rate, was calculated by dividing the number of negative cases

TABLE 1. Summary of participant information from three centers.

Parameter	PI-US		I-SE		UCK-PL	
	RT-PCR positive (n=44)	RT-PCR negative (n=247)	RT-PCR positive (n=100)	RT-PCR negative (n=114)	RT-PCR positive (n=223)	RT-PCR negative (n=302)
Age in years, mean (SD)	34.6 (18.9)	33.3 (17.5)	-	-	47.8 (21.7)	46.2 (24.5)
Gender (%)						
Male	20.0 (45.5)	99.0 (40.1)	-	-	115.0 (51.6)	144.0 (47.8)
Female	24.0 (54.5)	148.0 (59.9)	-	-	108.0 (48.4)	158.0 (52.3)
Cycle threshold (%)						
<15	0 (0)		1.0 (1.0)		0 (0)	
15 to <20	5.0 (11.4)		6.0 (6.0)		178.0 (79.8)	
20 to <25	12.0 (27.3)		8.0 (8.0)		15.0 (6.7)	
25 to <30	20.0 (45.5)		29.0 (29.0)		10.0 (4.5)	
30 to <35	7.0 (15.9)		56.0 (56.0)		20.0 (9.0)	
\geq 35	0 (0)		0 (0)		0 (0)	

Note: “-” means information not recorded.

Abbreviation: PI-US=Phamatech, Inc. in USA; I-SE=iLab in Sweden; UCK-PL=Uniwersyteckie Centrum Kliniczne in Poland; RT-PCR=real-time reverse transcription-polymerase chain reaction; SD=standard deviation.

identified by the ALLtest by the total number of true negative cases. The 95% confidence intervals (CIs) were derived using exact binomial tests.

To determine factors strongly correlated with antigen test outcomes, participants were divided into two categories according to their test results. Factors in each category were then compared for significance. For categorical variables such as sex and Ct category, Pearson's chi-square test was utilized. For continuous variables including age, symptom duration, and Ct value — depending on their distribution — either the T-test or the Wilcoxon test was employed. All statistical analyses were performed using R software (version 3.6.0, R Core Team, Vienna, Austria).

RESULTS

Preparation and Characterization of mAbs Against N Protein

The PCR amplification of the SARS-CoV-2 N gene resulted in a 1,200 bp band, as observed through 1% agarose gel electrophoresis (Figure 1A). Following immunization and cell fusion, 12 mAbs were produced against the SARS-CoV-2 N protein (Figure 1B). The titers for five of these mAbs were notably high, approximately 1:102,400, whereas the titers for the remaining mAbs were around 1:25,600 (Figure 1C). Subsequent to pair-wise screening (Figure 1D), mAbs N1, N12, N15, N16, and N17 were chosen for further analysis to identify antibody subtypes and develop the Ag-RDT Assay, due to their effective pairing performance and elevated titers. Notably, N16 and N17 were classified as IgM, while the other mAbs were identified as IgG1 subtype (Figure 1E).

The Assessment of the Sensitivity and Specificity of the Ag-RDT Assay

The Ag-RDT assay was constructed using paired mAbs based on the standard schematic for colloidal gold test strip assembly, as illustrated in Figure 2A. One mAb was conjugated with colloidal gold while its counterpart served as the capture line (T-line) antibody. Sensitivity varied across different antibody pairs when assessing both positive and negative samples. The assay achieved maximal detection sensitivity and specificity when colloidal gold was conjugated with N17 and the T-line utilized N16, as shown in Figure 2B. Testing with the optimized Ag-RDT assay on virus tissue culture and recombinant

SARS-CoV-2 N protein antigen at varying dilutions identified the lowest detection limits: 100 TCID₅₀/mL for heat-inactivated SARS-CoV-2 virus (Figure 2C) and 0.1 ng/mL for the recombinant N antigen.

To verify the specificity of the Ag-RDT assay, we tested it against four other human coronaviruses and 13 viruses that cause respiratory illnesses. The results, which showed no cross-reactions (Supplementary Table S1, available at <https://weekly.chinacdc.cn/>), confirmed the assay's specificity for detecting the SARS-CoV-2 antigen. Additionally, various substances including budesonide nasal spray, mucin, dexamethasone, flunisolide, mupirocin, oxymetazoline, phenylephrine, rebetol, relenza, tamiflu, tobramycin, and whole blood were demonstrated not to interfere with the Ag-RDT results at the concentrations used (Supplementary Table S2, available at <https://weekly.chinacdc.cn/>).

Performance Evaluation of the Ag-RDT Assay with SARS-CoV-2 Variants

To assess the detection limit of the Ag-RDT assay for various SARS-CoV-2 variants, including Alpha, Beta, Gamma, Delta, and Omicron, each variant was subjected to testing. Results presented in Supplementary Table S3 (available at <https://weekly.chinacdc.cn/>) demonstrate that the Ag-RDT assay can effectively detect SARS-CoV-2 variants such as B.1.1.7, B.1.351, B.1.617.3, P.1.2, B.1.617.2, B.1.1.529, BA.2, BA.4, and BA.5, down to specific dilution rates and concentrations.

The Clinical Validation of the Ag-RDT Assay

The ALLtest antigen test was assessed across three centers, as detailed in Table 2. Within the PI-US cohort of 291 participants, the ALLtest correctly identified 41 out of 44 confirmed cases and showed no false positives among excluded cases, achieving a sensitivity of 93.18% and a specificity of 100%. Similarly, the test demonstrated high clinical efficacy in the I-SE and UCK-PL cohorts, with sensitivities of 100% and 97.31%, and specificities of 100% and 99.67%, respectively. Notably in the PI-US center, the sensitivity increased to 97.30% (95% CI: 85.84%, 99.93%) for samples with Ct values below 30, and to 96.77% (95% CI: 83.30%, 99.92%) for patients displaying symptoms within the first week. Additionally, the UCK-PL center reported a higher

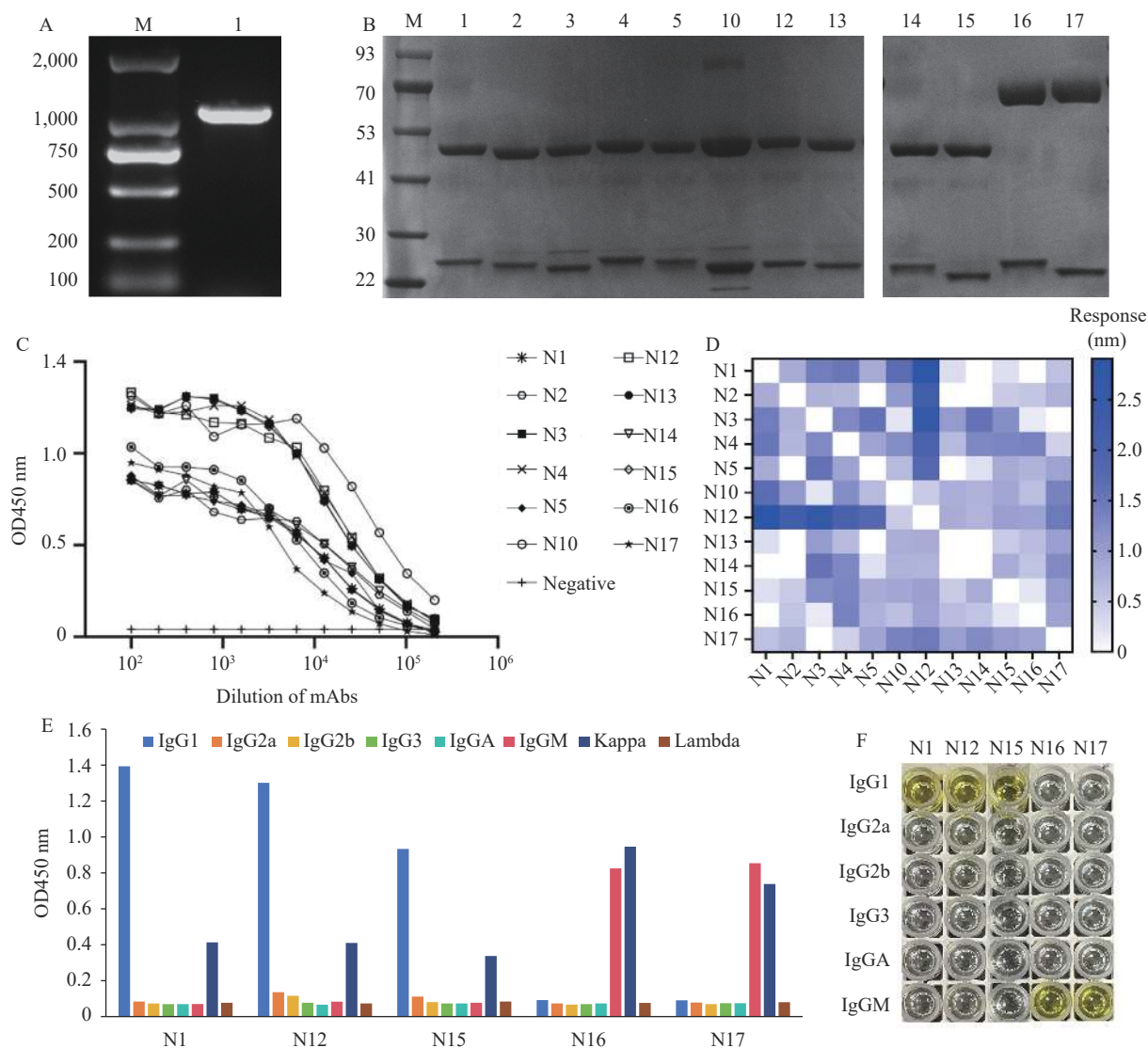


FIGURE 1. Characterization and paired screening of mAbs against the N protein. (A) Electrophoresis analysis of PCR products from pET-28a (+)-N. M: marker, L1: SARS-CoV-2 N gene; (B) SDS-PAGE analysis of SARS-CoV-2 N mAbs. M: marker, L1-L17: purified mAbs of SARS-CoV-2 N; (C) Titer detection of mAbs; (D) Heat map presenting paired results of mAbs; (E) Histogram of ELISA identification of mAbs subtypes N1, N12, N15, N16, and N17; (F) Identification of SARS-CoV-2 N mAbs subtypes using a monoclonal antibody subtyping kit.

Abbreviation: PCR=polymerase chain reaction; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SDS-PAGE=sodium dodecyl sulfate polyacrylamide gel electrophoresis; ELISA=enzyme linked immunosorbent assay.

sensitivity of 99.51% (95% CI: 97.29%, 99.99%) for samples with Ct values below 30.

Factors Correlated with Our Antigen Results

Clinical factors including age, sex, duration of symptoms, and Ct values of participants were assessed for correlations with results from the ALLtest antigen assay, as shown in [Supplementary Table S4](#) (available at <https://weekly.chinacdc.cn/>). Across three centers, results were consistent. A significant number of participants

exhibited low viral loads that precluded RNA detection by PCR, regardless of the number of amplification cycles performed. Therefore, artificial cutoff values were employed to categorize Ct values. In PI-US and UCK-PL settings, both actual Ct values (Figure 3A and 3B; $P=0.026$, $P=9.74\times 10^{-5}$) and categorized Ct values ($Ct<35$ versus $Ct\geq 35$; $P<2.2\times 10^{-16}$) demonstrated a significant correlation with antigen test outcomes. The timing of symptom onset correlated with antigen results exclusively in UCK-PL ($P=0.0018$; Figure 3C). No significant correlations between age or sex and antigen test results were identified.

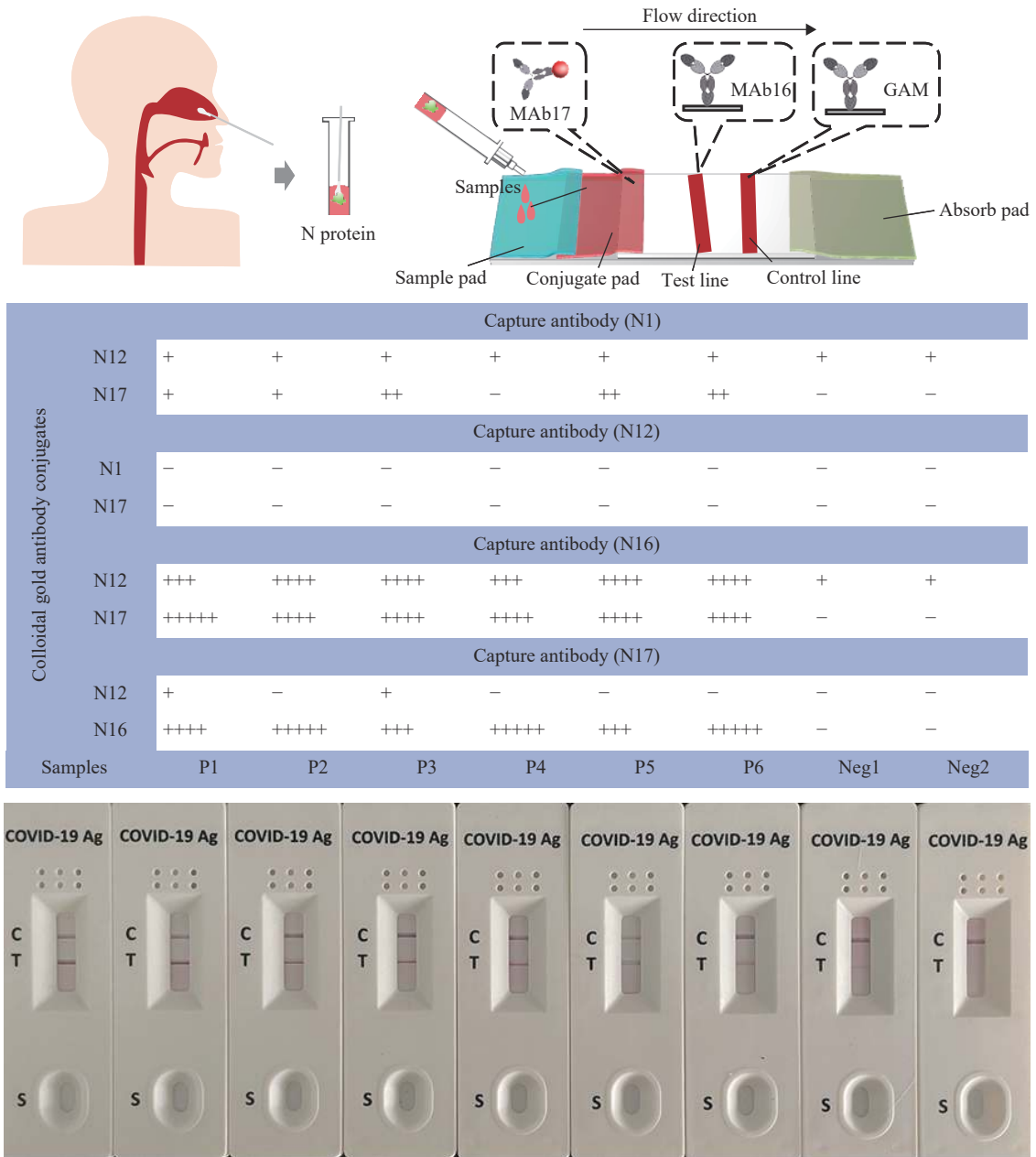


FIGURE 2. Sensitivity detection of the Ag-RDT assay for COVID-19. (A) The principal diagram of the colloidal gold test strip assembly and sample detection process; (B) Detection sensitivity and specificity using colloidal gold labeling and T-line labeling with various paired monoclonal antibodies (mAbs); (C) Sensitivity detection of the Ag-RDT assay for the heat-inactivated SARS-CoV-2 virus.

Note: In panel C, Lines 1–9 indicate concentrations ranging from 10^9 to 10 TCID₅₀/mL; Line 8 indicates that the minimal limit of detection is 100 TCID₅₀/mL.

Abbreviation: Ag-RDT=antigen early rapid diagnostic test; COVID-19=coronavirus disease 2019; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; TCID=tissue culture infectious doses.

DISCUSSION

The SARS-CoV-2 pandemic continues to pose significant challenges, necessitating effective strategies to curb its spread. Antigen testing has been crucial in early detection, containment, and the reduction of

transmission rates. Furthermore, its integration with the One Health approach emphasizes the interconnection of human, animal, and environmental health, highlighting the need for a robust and unified global health framework. Recent literature indicates varying findings regarding the application of antigen

TABLE 2. Sensitivity and specificity of ALLtest.

Antigen test results	PI-US			I-SE			UCK-PL		
	+	-	Total	+	-	Total	+	-	Total
Positive	41	0	41	100	0	100	217	1	218
Negative	3	247	250	0	114	114	6	301	307
Total	44	247	291	100	114	214	223	302	525
Test evaluation, % (95% CI)									
Sensitivity	93.2 (81.3, 98.6)			100.0 (96.4, 100.0)			97.3 (94.2, 99.0)		
Specificity	100.0 (98.5, 100.0)			100.0 (96.8, 100.0)			99.7 (98.2, 100.0)		

Note: “-” means negative. “+” means positive.

Abbreviation: PI-US=Phamatech, Inc. in USA; I-SE=iLab in Sweden; UCK-PL=Uniwersyteckie Centrum Kliniczne in Poland; RT-PCR=real-time reverse transcription-polymerase chain reaction; CI=confidence interval.

tests in diagnosing COVID-19. A meta-analysis of antigen tests developed before September 30, 2020, showed that under specific conditions, despite varied sensitivity, rapid antigen tests could serve as alternatives to RT-PCR (13). Conversely, Greub et al. reported mixed performances among 30 antigen tests, advocating for comprehensive validation before official adoption (8). Similarly, while Navero-Castillejos et al. recommended using antigen tests solely for identifying highly infectious individuals (10), Regev-Yochay et al. observed high sensitivity across symptomatic and asymptomatic patients alike (14). Kohmer et al. also noted a significant correlation between antigen test outcomes and cell culture infectivity, underscoring their potential utility in managing COVID-19 (9).

Adding to this body of work, we employed our Ag-RDT (ALLtest) in a substantial cohort across three international centers. Our findings revealed high clinical sensitivities (93.18%–100%) and specificities (99.67%–100%) in suspected COVID-19 cases when compared to RT-PCR. This outperformed six other commercial rapid antigen tests, which showed sensitivities ranging from 65% to 79% (10). According to WHO standards — a sensitivity of $\geq 80\%$ and specificity of $\geq 97\%$ — our ALLtest demonstrated excellent efficacy in all three centers, meeting the criteria for SARS-CoV-2 detection. However, historical data revealed that ALLtest was grouped among those with low sensitivity (16.7%) in a review of 122 Ag-RDTs by Nubling et al. (15). Nonetheless, recent studies, such as those by Moons et al. and Sivro et al., show more promising results, with higher sensitivities observed in nasal versus saliva samples (83.9%) and reasonable detection (about 74%) of Omicron BA.4 and BA.5 variants (16–17). Overall, the ALLtest exhibits generally qualified performance amid evolving epidemiological landscapes.

The Ct value, indicative of the quantity of viral RNA, has been recommended for inclusion alongside binary RT-PCR outcomes to enhance their clinical utility (18). Bullard et al. observed that no viable viral cultures were produced from samples with a Ct value greater than 24 (19). Additionally, studies have shown that samples with a Ct value exceeding 30 (2.17×10^5 E gene copies/mL) are typically not cultivable (20). The sensitivity of the ALLtest correlates with viral loads, consistent with previous findings (21–22). Similar to other research (14), the sensitivity of ALLtest was higher in samples with a Ct value of 30 or less (99.30%, 95% CI: 97.48%, 99.91%) and increased to 100% (95% CI: 98.38%, 100%) in samples with a Ct value of 25 or less. Significantly lower Ct values were also observed in ALLtest-positive cases among participants with detectable Ct values across all three centers (Figure 3A, 3B, and Supplementary Table S4). According to Kucirka et al., RT-PCR sensitivity and viral load typically increase within 5–7 days post-infection, stabilize for 1–2 weeks, and subsequently decrease (4). Furthermore, a significant difference in the timing of symptoms was found between ALLtest positive and negative groups, with those displaying symptoms earlier (2.25 days vs. 2.64 days) more likely to test positive with ALLtest ($P=0.0018$).

In this study, we exclusively utilized NPS for both Ag-RDT and RT-PCR. This limitation may affect the generalizability of the results, as the performance of the Ag-RDT could vary with other sample types, such as oropharyngeal swabs or saliva. And due to the difficulty of collecting enough number of participants infected by the recent coronavirus strain, we only validated the detection effect of ALLtest on those strains in lab. In the future, multiple commonly used Ag-RDT will be tested compared with ALLtest on a larger COVID-19-infected population.

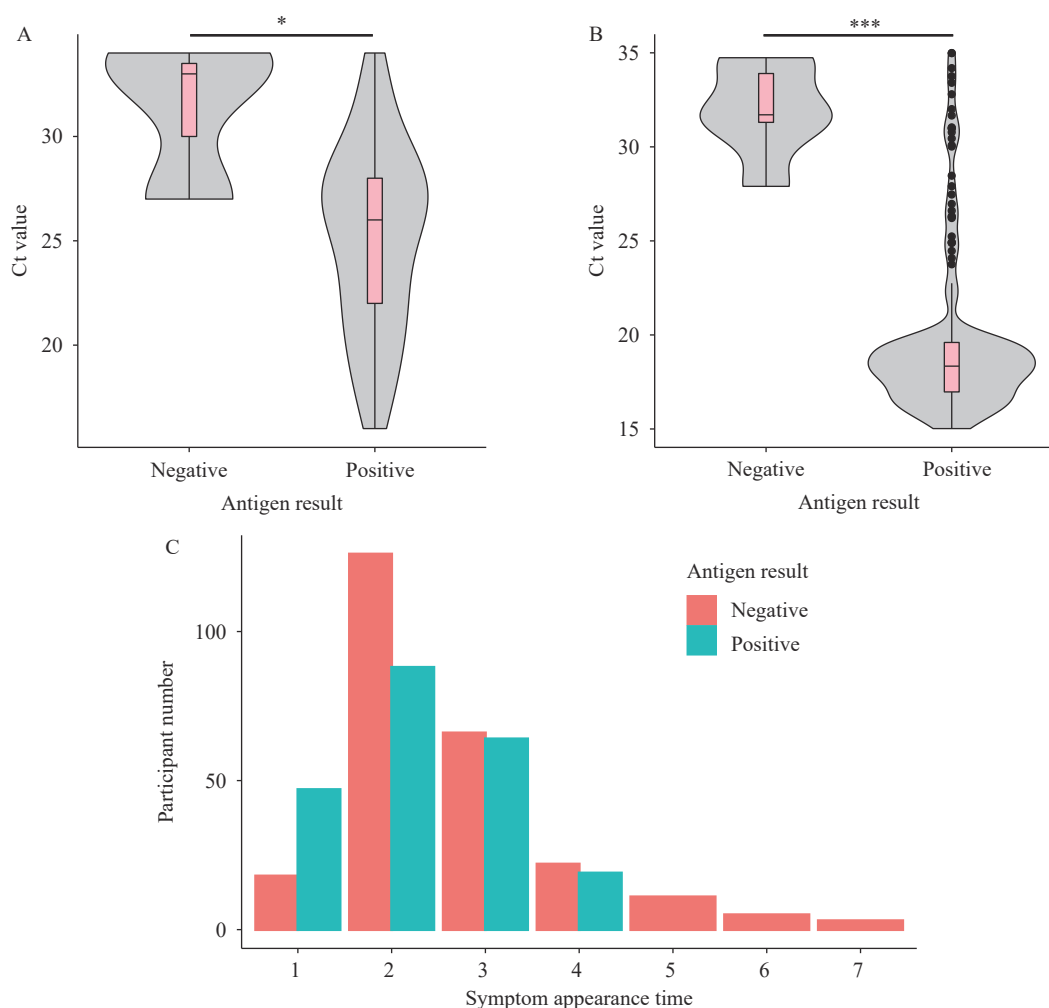


FIGURE 3. Correlation factors associated with ALLtest results. (A) Ct value distributions among participants from PI-US categorized by differing antigen test results ($P=0.026$); (B) Ct value distributions among participants from UCK-PL categorized by differing antigen test results ($P=9.74 \times 10^{-5}$); (C) Distributions of symptom onset among participants from UCK-PL with varying antigen test outcomes ($P=0.0018$).

Abbreviation: Ct=cycle threshold; UCK-PL=Uniwersyteckie Centrum Kliniczne in Poland; PI-US=Phamatech, Inc. in USA.

We developed and validated the ALLtest antigen test for the early diagnosis of COVID-19 across three international centers. Given its high sensitivity and specificity, we propose that the ALLtest can serve as a reliable adjunct to the RT-PCR test.

Conflicts of interest: No conflicts of interest.

Funding: Supported by the Zhejiang Provincial Program for the Cultivation of High-level Innovative Health Talents (WJW2021002); Science Foundation of National Health Commission of the PRC (WJW-ZJ-2203); the Key R&D projects of Zhejiang Province (2022C03109); the S&T Major Project of Lishui (2023GYX03) and the National Health Commission Research fund (WKJ-ZJ-2421).

doi: 10.46234/ccdcw2024.199

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Submitted: August 27, 2023; Accepted: September 08, 2024

REFERENCES

- World Health Organization. Weekly epidemiological update on covid-19 - 16 March 2023. 2023. <https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19--16-march-2023>. [2024-3-18].
- Shrestha LB, Foster C, Rawlinson W, Tedla N, Bull RA. Evolution of the SARS-CoV-2 omicron variants BA. 1 to BA.5: implications for immune escape and transmission. *Rev Med Virol* 2022;32(5):e2381. <https://doi.org/10.1002/rmv.2381>.
- Asadi S, Bouvier N, Wexler AS, Ristenpart WD. The coronavirus pandemic and aerosols: does COVID-19 transmit via expiratory particles?. *Aerosol Sci Technol* 2020;54(6):635 – 8. <https://doi.org/10.1080/02786826.2020.1749229>.
- Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. *Ann Intern Med* 2020;173(4):262 – 7. <https://doi.org/10.7326/M20-1495>.
- Brummer LE, Katzenschlager S, Gaeddert M, Erdmann C, Schmitz S, Bota M, et al. Accuracy of novel antigen rapid diagnostics for SARS-CoV-2: a living systematic review and meta-analysis. *PLoS Med* 2021;18(8):e1003735. <https://doi.org/10.1371/journal.pmed.1003735>.
- Khalid MF, Selvam K, Jeffry AJN, Salmi MF, Najib MA, Norhayati MN, et al. Performance of rapid antigen tests for COVID-19 diagnosis: a systematic review and meta-analysis. *Diagnostics (Basel)* 2022;12(1):110. <https://doi.org/10.3390/diagnostics12010110>.
- Ye Q, Shao WX, Meng HY. Performance and application evaluation of SARS-CoV-2 antigen assay. *J Med Virol* 2022;94(8):3548 – 53. <https://doi.org/10.1002/jmv.27798>.
- Greub G, Caruana G, Schweitzer M, Imperiali M, Muigg V, Risch M, et al. Multicenter technical validation of 30 rapid antigen tests for the detection of SARS-CoV-2 (VALIDATE). *Microorganisms* 2021;9(12):2589. <https://doi.org/10.3390/microorganisms9122589>.
- Kohmer N, Toptan T, Pallas C, Karaca O, Pfeiffer A, Westhaus S, et al. The comparative clinical performance of four SARS-CoV-2 rapid antigen tests and their correlation to infectivity in vitro. *J Clin Med* 2021;10(2):328. <https://doi.org/10.3390/jcm10020328>.
- Navero-Castillejos J, Casals-Pascual C, Narváez S, Cuesta G, Hurtado JC, Fernandez M, et al. Diagnostic performance of six rapid antigen tests for SARS-CoV-2. *Microbiol Spectr* 2022;10(2):e02351 – 21. <https://doi.org/10.1128/spectrum.02351-21>.
- Wu C, Qavi AJ, Hachim A, Kavian N, Cole AR, Moyle AB, et al. Characterization of SARS-CoV-2 nucleocapsid protein reveals multiple functional consequences of the C-terminal domain. *iScience* 2021;24(6):102681. <https://doi.org/10.1016/j.isci.2021.102681>.
- Pu YY, Weng YH, Wu YH, Gao F, Zheng XJ, Xiong XQ, et al. Antibody response to SARS-CoV-2 in the first batch of COVID-19 patients in China by a self-developed rapid IgM-IgG test. *Front Cell Infect Microbiol* 2022;12:915751. <https://doi.org/10.3389/fcimb.2022.915751>.
- Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst Rev* 2020;8(8):CD013705. <https://doi.org/10.1002/14651858.CD013705>.
- Regev-Yochay G, Kriger O, Mina MJ, Beni S, Rubin C, Mechnik B, et al. Real world performance of SARS-CoV-2 antigen rapid diagnostic tests in various clinical settings. *Infect Control Hosp Epidemiol* 2022;1-20. <http://dx.doi.org/10.1017/ice.2022.3>.
- Scheiblaue H, Filomena A, Nitsche A, Puyskens A, Corman VM, Drost C, et al. Comparative sensitivity evaluation for 122 CE-marked rapid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021. *Euro Surveill* 2021;26(44):2100441. <https://doi.org/10.2807/1560-7917.ES.2021.26.44.2100441>.
- Schuit E, Venekamp RP, Veldhuijzen IK, van den Bijllaardt W, Pas SD, Stohr JJJM, et al. Head-to-head comparison of the accuracy of saliva and nasal rapid antigen SARS-CoV-2 self-testing: cross-sectional study. *BMC Med* 2022;20(1):406. <https://doi.org/10.1186/s12916-022-02603-x>.
- Samsunder N, Lustig G, de Vos M, Ngcapu S, Giandhari J, Tshiabuila D, et al. Performance of rapid antigen tests in identifying Omicron BA. 4 and BA.5 infections in South Africa. *J Clin Virol* 2023;165:105498. <https://doi.org/10.1016/j.jcv.2023.105498>.
- Tom MR, Mina MJ. To interpret the SARS-CoV-2 test, consider the cycle threshold value. *Clin Infect Dis* 2020;71(16):2252 – 4. <https://doi.org/10.1093/cid/ciaa619>.
- Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. *Clin Infect Dis* 2020;71(10):2663 – 6. <https://doi.org/10.1093/cid/ciaa638>.
- Iglói Z, Velzing J, van Beek J, van de Vijver D, Aron G, Ensing R, et al. Clinical evaluation of Roche SD biosensor rapid antigen test for SARS-CoV-2 in municipal health service testing site, the Netherlands. *Emerg Infect Dis* 2021;27(5):1323 – 9. <https://doi.org/10.3201/eid2705.204688>.
- Jääskeläinen AE, Ahava MJ, Jokela P, Szivovicza L, Pohjala S, Vapalahti O, et al. Evaluation of three rapid lateral flow antigen detection tests for the diagnosis of SARS-CoV-2 infection. *J Clin Virol* 2021;137:104785. <https://doi.org/10.1016/j.jcv.2021.104785>.
- Smith RD, Johnson JK, Clay C, Girio-Herrera L, Stevens D, Abraham M, et al. Clinical evaluation of Sofia rapid antigen assay for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among emergency department to hospital admissions. *Infect Control Hosp Epidemiol* 2022;43(8):968 – 73. <https://doi.org/10.1017/ice.2021.281>.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Verification of specificity for viruses associated with respiratory infections.

No.	Virus strains	Concentration (TCID ₅₀ /mL)	Result
1	HCoV-229E	5×10 ⁵	-
2	HCoV-HKU1	1×10 ⁶	-
3	HCoV-OC43	1×10 ⁶	-
4	HCoV-NL63	1×10 ⁶	-
5	Parainfluenza viruses 2	1.58×10 ⁷	-
6	Parainfluenza viruses 3	1.58×10 ⁸	-
7	Influenza A (H3N2)	1×10 ⁵	-
8	Influenza A (H1N1)	3.16×10 ⁵	-
9	Influenza B	3.16×10 ⁶	-
10	Adenovirus type 3	3.16×10 ⁴	-
11	Adenovirus type 7	1.58×10 ⁵	-
12	Human Rhinovirus 2	2.81×10 ⁴	-
13	Human Rhinovirus 14	1.58×10 ⁶	-
14	Human Rhinovirus 16	8.89×10 ⁶	-
15	Measles virus	1.58×10 ⁴	-
16	Mumps virus	1.58×10 ⁴	-
17	Respiratory syncytial virus	8.89×10 ⁴	-

Note: “-” means negative.

SUPPLEMENTARY TABLE S2. Results of the interfering action by the Ag-RDT assay.

No.	Analytes	Concentration	Negative	SARS-CoV-2 positive
1	budesonide nasal spray	200 µL/mL	-	+
2	whole blood	20 µL/mL	-	+
3	relenza	282 ng/mL	-	+
4	flunisolide	6.8 ng/mL	-	+
5	mucin	50 µg/mL	-	+
6	rebetol	4.5 µg/mL	-	+
7	tamiflu	1.1 µg/mL	-	+
8	mupirocin	12 mg/mL	-	+
9	phenylephrine	12 mg/mL	-	+
10	tobryamycin	2.43 mg/mL	-	+
11	dexamethasone	0.8 mg/mL	-	+
12	oxymetazoline	0.6 mg/mL	-	+

Note: “-” means negative. “+” means positive.

Abbreviation: Ag-RDT=antigen early rapid diagnostic test; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

SUPPLEMENTARY TABLE S3. Detection of SARS-CoV-2 variants using the Ag-RDT assay.

WHO label	Pango lineages	N protein mutations	LOD
Alpha	B.1.1.7	D3L, R203K, G204R, S235F	1:2×10 ⁶
Beta	B.1.351	T205I	1:2×10 ⁶
VUI-21ARP-03	B.1.617.3	P67S, R203M, D377Y	1:2×10 ⁶
Gamma	P.1.2	P80R	1:2×10 ⁶
Delta	B.1.617.2	D63G, R203M, D377Y	1:2×10 ⁶
Omicron	B.1.1.529	P13L, del31/33, R203K, G204R	1:1×10 ⁶ (1.61 ng/mL)
Omicron	BA.2	P13L, del31/33, R203K, G204R	1:1×10 ⁷ (0.368 ng/mL)
Omicron	BA.4	P13L, del31/33, P151S, R203K, G204R, S413R	1:1×10 ⁷ (0.29 ng/mL)
Omicron	BA.5	P13L, del31/33, R203K, G204R, S413R	1:1×10 ⁷ (0.368 ng/mL)

Note: LOD indicated by the highest dilution rate of the virus that can be detected.

Abbreviation: Ag-RDT=antigen early rapid diagnostic test; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; LOD=limit of detection; WHO=World Health Organization.

SUPPLEMENTARY TABLE S4. Correlation significance of factors across three centers.

P	PI-US	I-SE	UCK-PL
Sex	0.55	–	0.29
Age	0.78	–	0.42
Symptom time	0.47	0.11	0.0018 [†]
Ct value	0.026 [†]	–	9.74×10 ^{-5†}
Ct category*	<2.2×10 ^{-16†}	<2.2×10 ^{-16†}	<2.2×10 ^{-16†}

“–” means significance cannot be calculated on missing data.

Abbreviation: PI-US=Phamatech, Inc. in USA; I-SE=iLab in Sweden; UCK-PL=Uniwersyteckie Centrum Kliniczne in Poland; Ct=cycle threshold.

* Ct category include: 1) Ct<35; 2) Ct≥35.

†: P<0.05.