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Evaluation of a high-sensitivity SARS-CoV-2 antigen test on the fully automated light-initiated chemiluminescent immunoassay platform

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Abstract

Objectives: To describe a high-sensitivity SARS-CoV-2 antigen test that is based on the fully automated light-initiated chemiluminescent immunoassay (LiCA®), and to validate its analytical characteristics and clinical agreement on detecting SARS-CoV-2 infection against the reference molecular test.

Methods: Analytical performance was validated and detection limits were determined using different types of nucleocapsid protein samples. 798-pair anterior nasal swab specimens were collected from hospitalized patients and asymptomatic screening individuals. Agreement between LiCA® antigen and real-time reverse transcription polymerase chain reaction (rRT-PCR) was evaluated.

Results: Repeatability and within-lab precision were 1.6–2.3%. The C_5~C_{95} interval was −5.1–4.6% away from C_{50}. Detection limits in average (SD) were 325 (±141) U/mL on the national reference panel, 0.07 (±0.04) $TCID_{50}$/mL on active viral cultures, 0.27 (±0.09) pg/mL on recombinant nucleocapsid proteins and 1.07 (±1.01) $TCID_{50}$/mL on inactivated viral suspensions, respectively. LiCA detected a median of 374-fold (IQR 137–643) lower levels of the viral antigen than comparative rapid tests. As reference to the rRT-PCR method, overall sensitivity and specificity were determined to be 97.5% (91.4–99.7%) and 99.9% (99.2–100%), respectively. Total agreement between both methods was 99.6% (98.7–99.9%) with Cohen's kappa 0.98 (0.96–1). A positive detection rate of 100% (95.4–100%) was obtained as Ct≤37.8.

Conclusions: The LiCA® system provides an exceptionally high-sensitivity and fully automated platform for the detection of the SARS-CoV-2 antigen in nasal swabs. The assay may have high potential use for large-scale population screening and surveillance of COVID-19 as an alternative to the rRT-PCR test.

Keywords: coronavirus disease 2019; high-sensitivity; LiCA®; light-initiated chemiluminescent assay; rRT-PCR; SARS-CoV-2.

Introduction

A global pandemic of coronavirus disease-2019 (COVID-19), caused by infection of severe acute respiratory syndrome coronavirus type-2 (SARS-CoV-2), has created a tremendous impact on economies and lives worldwide [1, 2]. Given unexpectable transmissibility of SARS-CoV-2 variants [3], early diagnosis and isolation of patients and their contacts remain a key strategy for control and management of this pandemic. Therefore, a highly sensitive and affordable detection approach for SARS-CoV-2 infection is essential in clinical practice.

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Nucleic acid-based molecular testing with real-time reverse transcription polymerase chain reaction (rRT-PCR) has been established as a "gold-standard" for COVID-19 diagnosis since the first wave of the outbreak [4, 5]. However, considerable disadvantages of RT-PCR such as high costs and long turn-around time raise substantial challenges when applied for a large-scale screening [6]. Rapid point of care antigen tests (RAT) can be used as an alternative for the diagnosis of active SARS-CoV-2 infection in a faster and less expensive way than molecular tests [7, 8]. However, lack of sensitivity [9, 10] may limit their use only for detection of individuals with high viral load. In addition, most RATs require a manual operation to perform the sample one by one. Absence of automatic processing and registration creates significant labor challenges during a large population screening program.

Recent studies have indicated that laboratory-based SARS-CoV-2 antigen tests, with high-volume and fully automated processing, may have advantages on detecting SARS-CoV-2, especially in a large-scale screening [11, 12]. High-volume antigen tests are primarily based on chemiluminescent technology and able to offer improved sensitivity compared to RATs [13]. Unfortunately, a majority of the high-volume assays available so far have been identified with inadequate sensitivity for early detection of COVID-19 [14, 15]. To overcome this problem, a more sensitive high-volume antigen assay is necessary.

In this study, we aim to describe a high-sensitivity SARS-CoV-2 antigen test that is based on the fully automated light-initiated chemiluminescent immunoassay (LiCA®), and to validate its analytical characteristics and clinical agreement on detecting SARS-CoV-2 infection against the reference molecular test.

Materials and methods

Study design

This work was designed to evaluate the analytical and clinical performance of the LiCA® SARS-CoV-2 antigen assay, primarily performed at the National Infectious Disease Medical Center of the Capital Medical University affiliated Beijing Ditan Hospital. The active viral culture experiments were carried out at the Biosafety Level-3 (BSL-3) laboratory of Chinese Center for Disease Control and Prevention (CDC). The study was approved by the Ethics Committee of the Hospital (No. SJ2022-015).

SARS-CoV-2 antigen assays

LiCA® SARS-CoV-2 antigen assay (Chemclin Diagnostics, Beijing, China) is a one-step sandwich chemiluminescent immunoassay on specifically detecting the nucleocapsid protein of SARS-CoV-2. The assay shares the same methodology as the luminescent oxygen channeling immunotechnology (LOCI) [16, 17] and is running on the fully automatic LiCA® serial analyzers. The assay specifically detects the nucleocapsid protein of SARS-CoV-2. A signal directly proportional to the antigen titer is recorded and translated into a cut-off index (COI). A positive result is considered as COI≥1.0. The LiCA® 500 system in this study uses disposable tips for sampling. No wash tubing is configured throughout the machine. The system is designed to perform the assay with a fully automatic running model in 24 h and 7 days and capable to report 250 results per hour. Time to the first result is within 25 min.

Comparative SARS-CoV-2 antigen tests include four commercial RATs from Acon (Hangzhou, China), Wondfo (Guangzhou, China), Wantai (Beijing, China) and Hotgen (Beijing, China), which are all commonly used tests based on lateral flow chromatography. These strips contain monoclonal antibodies specific to the SARS-CoV-2 nucleocapsid protein. Results are read with negative or positive report in 10–30 min. Detection limits of Acon, Wondfo and Wantai are claimed to be 160, 850 and 137 $TCID_{50}$/mL, respectively. Hotgen doesn't announce its detection limit value. All assays were conducted by one experienced technician according to the manufacturer's protocols. Results were recorded by the operator and then double confirmed by another two well-trained personnel independently.

SARS-CoV-2 molecular testing (rRT-PCR)

SARS-CoV-2 molecular testing was performed by well-experienced personnel from the clinical laboratory of the hospital, strictly following the manufacturer's instructions. Total nucleic acid was isolated from nasal swabs in the viral transport medium (VTM) with a DaAn Gene nucleic acid extraction kit (DaAn Gene, Guangzhou, China). The rRT-PCR assay was performed by using a DaAn Gene 2019-nCoV detection kit on the Gentier 96E/96R Real-time PCR system (Tianlong, Xi'an, China). Cycle threshold (Ct) values were determined by targeting the viral ORF1ab and N genes. The detection limit is 200 virus copies/mL as claimed. Measurements with Ct≤40 are considered as positive.

Analytical validation

Precision was evaluated following the protocol of Clinical and Laboratory Standards Institute (CLSI) EP5-A3 [18]. Acceptance was considered as percent coefficient of variation (CV%) <10%. Additional C_{50} tests were performed using negative and positive controls (LiCA®, Lot 2201) according to the CLSI EP12-A2 guidelines [19]. A maximum of C_5~C_{95} interval within $C_{50} \pm 20\%$ was regarded as acceptable.

Detection limit of the LiCA® assay was determined and compared with four RATs using four types of controls: (1) the reference panel for SARS-CoV-2 antigen detection from National Institutes for Food and Drug Control (NIFDC, Lot 370095-202202). (2) two strains of active viral cultures from China CDC, containing 1,000 $TCID_{50}$/mL of Delta and Omicron variants, respectively. (3) three tubes of recombinant nucleocapsid proteins of SARS-CoV-2 (A, national reference material by Chinese National Institute of Metrology, Cat. GBW 091097; B, commercial material, HeavyBio, Shenzhen, China, Cat. HP811-50; and C, commercial material, Fapon Biotech, Dongguan, China, Cat. nCoV-PS-Ag6). (4) five strains of inactivated viral suspensions from Bioantibody Biotechnology Co.,Ltd (Nanjing, China), containing 1,000 $TCID_{50}$/mL of Prototype, Beta, Gamma, Delta and Omicron variants, respectively. Each sample was

diluted into a serial of suspensions as the product instructions. Replicate assays were performed for each dilution in parallel by LiCA and four comparative RATs. To determine the detection limit in more precise, we repeated the targeting dilution for 20 times. A final decision was made as the number of positive results was no less than 19 (≥95%). Moreover, we carried out the rRT-PCR testing on the active viral dilutions and correlated Ct values with antigen assay COIs.

A further study was conducted to evaluate assay detection capability to different source of SARS-CoV-2 antigens and cross reactivity from other pathogens using the national reference control panel. The controls were from NIFDC (Lot 370095-202202) and composed of 8 positive and 20 negative inactivated suspensions. The positive samples contain different source of SARS-CoV-2 and the negative ones contain 20 potentially interfering pathogens, such as coronavirus OC43 and avian influenza virus H7N9 (Supplementary Table 1).

Agreement evaluation in clinical samples

We recruited 798 patients for collection of nasal swab specimens in our hospital between April and July, 2022, including COVID-19 in-patients 48 (6.0%) and contacts 51 (6.4%), and asymptomatic screening 699 (87.6%). Among of 798 subjects, 716 (89.7%) were confirmed to be PCR-negative and 82 (10.3%) PCR-positive. The negative group median age was 32 (interquartile range: IQR, 27–38) years and female was 445 (62.2%). Regarding 82 PCR-positive individuals, median age was 35 (IQR, 25–40) years and female was 28 (34.1%). 15 positive cases (18.3%) were detected on abroad visitors. 63 out of 82 (76.8%) were observed with COVID-19 related symptoms and time to sampling from symptomatic onset was median 5 (IQR 3–7) days. Detail information of virus mutations was unknown but Omicron BA.2 and BA.5 variants were identified in the positive group. All patients were swabbed with paired-nasal specimens in parallel. Samples were stored in each VTM specific for testing of either LiCA® antigen or rRT-PCR and assayed within 4 h after collection. Clinical agreement between two methods was evaluated upon COI and Ct values recorded.

Statistics

LiCA® antigen tests were evaluated upon COI values with positive (≥1.0) or negative (<1.0). The molecular testing results were used as reference to calculate assay sensitivity and specificity. A statistical significance was considered as p-value <0.05. All data were analyzed by the software program MedCalc (MedCalc Software, Mariakerke, Belgium), OriginPro (OriginLab, MA, USA) and Excel (Microsoft, WA, USA).

Results

Precision analysis

According to the EP5-A3 protocol, we used COI values to assess the assay repeatability and intermediate precision. The results obtained for the low and high values were 1.60–2.30% by the LiCA® SARS-CoV-2 antigen assay (Table 1). Furthermore, the C_{50} imprecision curve of the assay were evaluate. The C_5~C_{95} interval was identified to be −5.1–4.6% away from C_{50} (Figure 1). The assay was confirmed to be with an excellent analytical precision per both quantitative and qualitative perspectives.

Analytical sensitivity and variant detection of the LiCA® SARS-CoV-2 antigen assay

To test analytical sensitivity and variant detection of the LiCA® SARS-CoV-2 antigen assay, we recorded detection limits of LiCA in comparison of four RATs upon different sources of proteins as shown in Figure 2. Using a vial of the national reference panel specific for the detection limit study of SARS-CoV-2 antigen, LiCA detected the viral antigen in average (SD: standard deviation) as low as 325 (±141) U/mL, which was median 576-fold (IQR 248–1,121) lower than that of comparative RATs (Figure 2A). Using active viral cultures (Figure 2B), LiCA detected the analyte down to 0.07 (±0.04) $TCID_{50}$/mL with a median of 521-fold (IQR 140–1,133) lower than comparative RATs. Similar findings were observed as shown in Figure 2C and D, in which the detection limits of LiCA were determined to be 0.27 (±0.09) pg/mL

Table 1: Precision analysis of the LiCA® SARS-CoV-2 antigen assay with EP5-A3 protocol.

Sample	Mean	Repeatability		Within-lab precision	
	(Cut-off index)	SD	CV%	SD	CV%
QC L	3.61	0.082	2.27	0.083	2.30
QC H	36.19	0.580	1.60	0.690	1.91

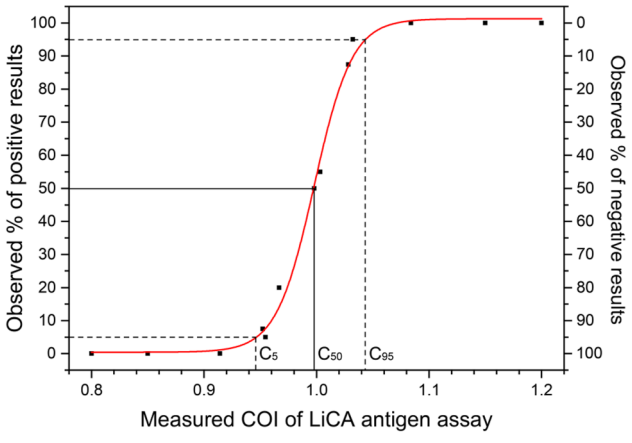

Figure 1: Evaluation of C_{50} imprecision curve of the LiCA® SARS-CoV-2 antigen assay using EP17-A2 protocol. A very steep curve was observed near C_{50}. The C_5~C_{95} interval was calculated to be −5.1–4.6% away from C_{50}.

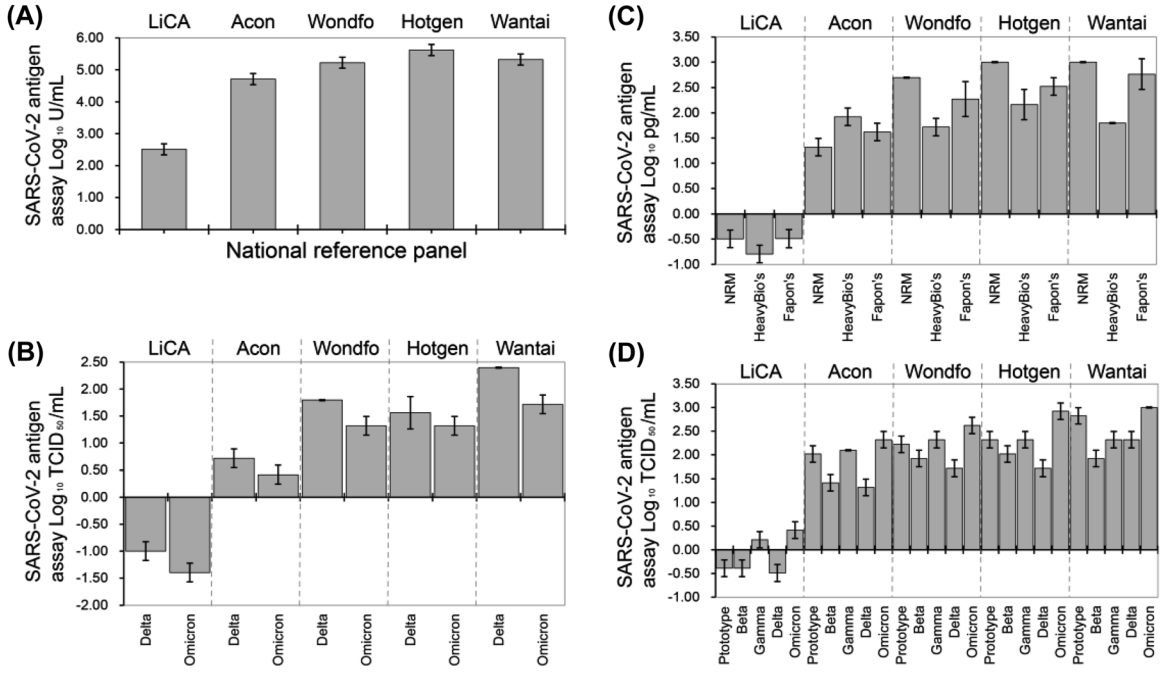

Figure 2: Comparison of detection limits on different measurands between LiCA® SARS-CoV-2 and rapid point of care antigen assays. Dilution serials were prepared according to the product instruction for following measurands: (A) national reference panel for SARS-CoV-2 antigen detection. (B) Active viral cultures. (C) Recombinant nucleocapsid proteins from national reference material (NRM), and productions of HeavyBio and Fapon. (D) inactivated viral suspensions. Each dilution was assayed in parallel by LiCA and comparative rapid antigen tests. The dilution targeting at the detection limit of each method was repeated for 20 times. A final decision was made as the number of positive results was no less than 19 (≥95%).

on recombinant proteins and 1.07 (±1.01) $TCID_{50}$/mL on inactivated viral suspensions. In overall, the detection capability of LiCA was estimated to be median 374-fold (IQR 137–643) better than the targeting comparisons based on the four types of measurands studied.

Diagnostic performance according Ct values

We subsequently performed a correlation analysis between LiCA® antigen assay COIs and viral load as determined by the Ct-values from the RT-qPCR diagnostics. The results show the relationship between LiCA® antigen assay COIs and rRT-PCR Ct values on dilution serials of active Omicron and Delta variant cultures (Table 2). Antigen COIs were tightly correlated to Ct values in reverse and Pearson's coefficients were determined to be −0.98~-0.99 on Log transformed COIs with Ct values across a broad range of viral concentration (0.06–1,000 $TCID_{50}$/mL). Notably, in lower levels of viral suspensions (0.03–0.12 $TCID_{50}$/mL), rRT-PCR may give a negative report while LiCA remained positive.

Cross-reactivity and interference

A national reference control panel was used to validate the assay accuracy. 8 positive controls with different source of SARS-CoV-2 antigens and 20 negative controls containing potentially interfering pathogens (Supplementary Table 1) were assayed by LiCA. All positive controls were detected with high values of COI (723–2,424) and negative ones were only recorded with noise signals (0.2–0.5). Therefore, the test exhibits nice specificity to SARS-CoV-2 antigens.

Assay agreement in clinical samples

To assess agreement between LiCA® antigen and rRT-PCR assays in clinical samples, 81 PCR-positive and 715 PCR-negative cases were included in the analysis (Table 3). Paired comparison of antigen results with rRT-PCR revealed that LiCA achieved an overall sensitivity of 97.5% (95% CI: confidence interval, 91.4–99.7%) and specificity of 99.9% (99.2–100%). Total agreement between both methods was 99.6% (98.7–99.9%) with Cohen's kappa 0.98 (0.96–1). A positive detection rate of 100%

Table 2: Relationship between LiCA® antigen and rRT-PCR results on the dilution serials of active virus cultures.

Dilution	Concentration	Omicron strain		Delta strain	
	$TCID_{50}$/mL	COI[a] mean (SD)	Ct[b] mean (SD)	COI mean (SD)	Ct mean (SD)
1	1,000.00	2,572.2 (214.8)	20.0 (1.7)	2,232.0 (180.2)	24.5 (1.7)
0.5	500.00	2,404.8 (221.0)	21.2 (1.9)	1,899.9 (181.2)	25.2 (1.8)
0.25	250.00	2,065.3 (172.5)	22.2 (1.7)	1,334.8 (72.4)	25.9 (2.0)
0.125	125.00	1,662.0 (203.5)	23.1 (1.9)	810.8 (72.4)	27.2 (2.2)
6.3×10^{-2}	62.50	1,160.5 (75.4)	24.8 (2.1)	416.6 (13.3)	28.4 (2.1)
3.1×10^{-2}	31.25	725.8 (70.2)	25.3 (2.3)	264.0 (16.3)	29.4 (2.2)
1.6×10^{-2}	15.63	368.8 (24.0)	26.3 (2.0)	189.1 (23.7)	31.0 (2.4)
7.8×10^{-3}	7.81	225.6 (25.9)	26.7 (2.4)	72.2 (6.8)	31.3 (2.3)
3.9×10^{-3}	3.91	101.3 (7.7)	27.0 (2.6)	36.5 (4.8)	32.4 (2.6)
2.0×10^{-3}	1.95	44.6 (3.5)	31.2 (2.8)	27.9 (0.4)	34.2 (2.3)
9.8×10^{-4}	0.98	23.4 (2.1)	29.8 (2.1)	5.7 (0.2)	35.2 (2.5)
4.9×10^{-4}	0.49	12.2 (1.3)	31.4 (2.5)	3.4 (0.3)	35.8 (3.2)
2.4×10^{-4}	0.24	5.8 (0.4)	33.3 (2.5)	1.9 (0.2)	36.8 (3.1)
1.2×10^{-4}	0.12	3.0 (0.3)	34.9 (3.1)	1.3 (0.2)	>40
6.1×10^{-5}	0.06	1.9 (0.2)	36.3 (3.3)	1.0 (0.1)	>40
3.1×10^{-5}	0.03	1.2 (0.2)	>40		

[a]COI, cut-off index. A positive result was considered as COI ≥1.0. [b]Ct, cycle threshold. A positive result was considered as Ct ≤40.

(95.4–100%) was observed in participants who had a Ct value ≤37.8. For 81 PCR-positive nasal swab specimens, LiCA® antigen COIs were assayed with median 1,534.5 (IQR 334.9–2,174.3) and range from 0.4–2,642.2 while rRT-PCR Ct values were recorded with median 27.5 (IQR 23.9–33.5) and range from 39.0–20.5. The log2-transformed antigen COIs showed a clear reverse correlation (r=−0.83) with Ct values (Figure 3).

Discussion

Antigen-detecting tests have been proposed as a viable screening tool for SARS-CoV-2 with advantages such as simple of use, shorter turn-around time and lower cost [8]. Unfortunately, less satisfactory sensitivity, whatever rapid point of care tests [9, 10] or high-volume assays [15, 20], constrains current antigen assays in detecting only for symptomatic cases but not for asymptomatic individuals [7]. Antigen assays detect surface proteins on the viral molecules and tell whether someone is infectious or in risk for potential transmissibility [8, 21]. A small amount of viral molecules during the early stage of infectious window may generate a false negative testing result [22, 23]. Missing pre-symptomatic individuals with COVID-19 may become the source of contagion, possibly triggering a regional pandemic. Thereby high sensitivity is an essential attribute to the antigen assay when applied for large scale screening.

Theoretically the LiCA® system may be capable of offering a highly sensitive assay. LiCA shares the same methodology as LOCI [16, 17] and uses nanoscale beads as the carriers of pre-coated reagent antibodies. The smaller particle of a nano-bead provides bigger specific surface area thus increasing pre-coated antibody density and detection capability to antigen. Improved sensitivity can be also obtained from remarkable signal amplification which was induced by the flow of 11,000/s singlet oxygen molecules from each sensitizer bead into the chemiluminescent beads [17]. Moreover, the binding kinetics showed that the streptavidin-biotin-labeled reaction provided a linear increasing signal with over five orders of magnitude for the LOCI assay [16]. Subsequent investigations have demonstrated that the LOCI HBsAg test not only detected 20-fold lower levels of analyte than the most contemporary assays but also remained more sensitive than the PCR method [16, 24]. The nature of exceptionally high sensitivity on LiCA® thyroid stimulating hormone and cardiac troponin I assays has been also illustrated by recent findings [25, 26].

In line with above, detection limits of the LiCA® SARS-CoV-2 antigen assay were determined herein to be average 325 (±141) U/mL on the national reference panel, 0.07 (±0.04) $TCID_{50}$/mL on active viral cultures, 0.27 (±0.09) pg/mL on recombinant nucleocapsid proteins and 1.07 (±1.01) $TCID_{50}$/mL on inactivated viral suspensions, which were median 374-fold (IQR 137–643) lower than those of comparative RATs. A further evaluation was carried out to testify the assay performance in clinical samples against the reference rRT-PCR testing. The overall sensitivity and specificity were 97.5 and 99.9%, respectively. A perfect detection rate of 100% can be obtained as Ct≤37.8. The active viral dilution study showed that LiCA COIs were tightly correlated to Ct values in reverse (R −0.98∼−0.99). This finding was in accordance with the previous report, in which the higher Ct value was identified to be related to the lower SARS-CoV-2 antigen level [27] and viral load [28, 29], thus predicting possibly decreased infectivity [28, 29]. LiCA recorded two false negative results throughout this study but they both were determined with

Table 3: Clinical agreement between LiCA® SARS-CoV-2 antigen and rRT-PCR assays.

rRT-PCR		LiCA® SARS-CoV-2 antigen				Agreement
Results	n	Positive	Negative	Sensitivity (95% CI)	Specificity (95% CI)	(95% CI)
Positive	81	79	2	97.5% (91.4–99.7%)	N/A[a]	97.5% (91.4–99.7%)
Ct[b]≤37.8	78	78	0	100% (95.4–100%)	N/A	100% (95.4–100%)
Negative	715	1	714	N/A	99.9% (99.2–100%)	99.9% (99.2–100%)
Total	796	80	716	97.5% (91.4–99.7%)	99.9% (99.2–100%)	99.6% (98.7–99.9%)

[a]N/A, not applicable. [b]Ct, cycle threshold.

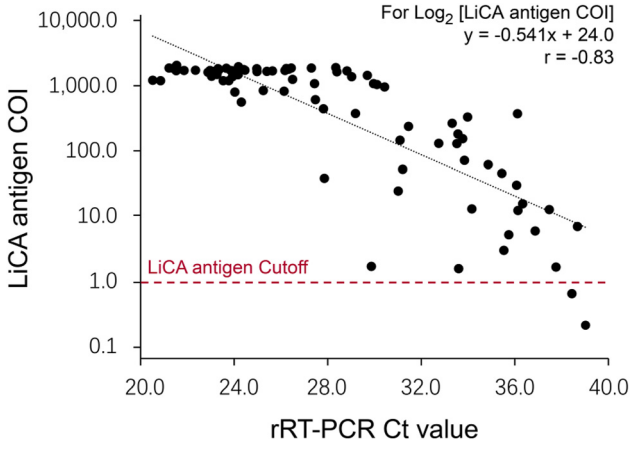

Figure 3: Correlation between LiCA® SARS-CoV-2 antigen assay COIs and cycle threshold (Ct) values of rRT-PCR on PCR-positive nasal swab specimens. The diagonal black dash line is the linear regression fit to Ct values for log_2-transformed antigen COIs. The LiCA® assay cutoff is marked with the horizontal red dash line. Two dots under the cutoff line indicate negative results while all data above cutoff are positive.

very high Ct values (>38). Interestingly, we observed another case that was identified to be strong positive (COI=175.3) by LiCA but negative by rRT-PCR on admission. However, rRT-PCR recovered a strong positive result (Ct=17.1) at the next day. The patient was diagnosed with COVID-19 finally. LiCA showed an earlier detection than rRT-PCR for this case. Detection in the lower levels of viral suspensions (0.03–0.12 $TCID_{50}$/mL) was also observed for LiCA rather than rRT-PCR on dilution serials of active Omicron and Delta variant cultures. A perfect sensitivity of 100% illustrated by LiCA even on a Ct value as high as 37.8 indicted that the assay may have a superior detection capability to early infection of SARS-CoV-2. This can be quite important on detecting asymptomatic individuals with COVID-19 and valuable to decrease the risk of transmission.

Besides of high sensitivity, full automation and high volume can be another advantage of the LiCA® assay for large population screening. The analyzer automatically performed assays with a walk-away model. Tests were programmed either in large-volume batch or any flexible tubes and supplied with intelligent barcodes. Sample information was registered through auto-scanning and results were promptly uploaded into the database once assay completed. Furthermore, the machine did not contain any wash-tubing. Thereby no water supply and waste drainage were necessary. This may award the assay highly favorable in a variety of settings upon demand of pandemic management, such as mobile cabin hospitals, community centers, medical vehicles, even aircraft carriers.

Although nasopharyngeal swabs have been proposed to be the primary choice for detection of SARS-CoV-2 [30], nasal swabs can be more applicable during a large-screening program. Nasal swabbing was more accessible and comfortable thus more acceptable for patients [31]. Also, it has been demonstrated that nasal swabs were qualified to offer a comparable assay with nasopharyngeal swabs for SARS-CoV-2 antigen [32].

There were limitations due to the small size of clinical samples and untailored sampling participants in this study. Herein we well demonstrated excellent detection capability of the assay and clinical agreement compared to rRT-PCR. A further study is necessary for evaluation of the real-world diagnostic performance for COVID-19 in a large-sized screening population with various types of subjects tailored upon different epidemic conditions and timing from symptomatic onset. Moreover, development of the assay in blood may be helpful, with a more standardized process, to provide a sensitive new marker for COVID-19 diagnosis and better discrimination of severity as an alternative to the molecular test [33–35].

In summary, the LiCA® system provides an exceptionally high-sensitivity and fully automated platform for detection of the SARS-CoV antigen in nasal swabs. During the early stage of exposure to the virus, both molecular tests and antigen assays could give a false negative report [22, 23]. The best strategy for the pandemic control is to run screening tests more frequently [8]. With great advantages on superior detection capability, excellent agreement with rRT-PCR, and cost-effectiveness, and washing-free benefits, the LiCA® assay may have a promising future in use for large-scale population

screening and surveillance of COVID-19 as an alternative of the expensive and cumbersome molecular test.

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Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interests.

Informed consent: Informed written consent was obtained from all participants before swab specimen collection for this study.

Ethical approval: Research involving human subjects complied with all relevant national regulation, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by Ethics Committee of the Capital Medical University affiliated Beijing Ditan Hospital (No. SJ2022-015).

Data availability: The datasets generated during the current study are available from the corresponding author on reasonable request.

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