Research Article

Multicenter evaluation of a simple and sensitive nucleic acid self-testing for SARS-CoV-2

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ABSTRACT

A rapid and accurate COVID-19 diagnosis is a prerequisite for blocking the source of infection as soon as possible and taking the appropriate medical action. Herein, we developed GeneClick, a device for nucleic acid self-testing of SARS-CoV-2, consisting of three modules: a sampling kit, a microfluidic chip-based disposable cartridge, and an amplification reader. In addition, we evaluated the clinical performance of GeneClick using 2162 nasal swabs collected at three medical institutions, using three commercial RT-qPCR kits and an antigen self-test as references. Compared to RT-qPCR, the sensitivity and specificity of the GeneClick assay were 97.93% and 99.72%, respectively, with a kappa value of 0.979 ($P < 0.01$). Of the 2162 samples, 2076 were also tested for SARS-CoV-2 antigens. Among the 314 positive samples identified by GeneClick assay, 63 samples were undetected by antigen tests. Overall, the GeneClick nucleic acid self-test demonstrated higher accuracy than the antigen-based detection. Based on the additional features, including simple operation, affordable price, portable device, and reliability of smartphone APP-driven sampling and result reporting, GeneClick offers a powerful tool for field-based SARS-CoV-2 detection in primary healthcare institutions or at-home use.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a significant public health burden since it was first reported in 2019 (Zhu et al., 2020a,b). An early, rapid, and accurate diagnosis is critical to isolate the source of infection as soon as possible and avoid a large-scale epidemic (Bi et al., 2020; Peeling et al., 2022). Outbreak management is hampered by high transmission rates and limitations in detection capacity, including early and rapid detection, which requires effective public health tools. The current gold standard for SARS-CoV-2 detection is the real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) (Tsang et al., 2021). However, its cost, long turnaround time, and the requirement for professional laboratory personnel and specialized laboratory equipment limit its application in multiple scenarios (Harmon et al., 2021). Although technological improvements in

the PCR amplification equipment, such as faster heating and cooling modules, have greatly reduced the amplification times, sample testing often take more than 6 h to receive a valid result due to the long queues at the nucleic acid testing sites.

Different screening setting modalities during the SARS-CoV-2 pandemic response marked the importance of delivering test results to patients before leaving the testing site. Precisely, to avoid a situation where the infected person continues to be active in society after sampling, field testing for SARS-CoV-2 infection needs to be rapid and performed at the sampling point, relying only on simple and portable equipment and operations (García-Bernalt Diego et al., 2022; Porte et al., 2020). With distinctive advantages of rapidity and simplicity, antigen testing is widely used in self-testing at home as a supplement to nucleic acid testing. However, antigen detection has limited specificity, sensitivity, and accuracy (Chen et al., 2022; Corman et al., 2021; Mak et al.,

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2020). In addition, antigen testing at home cannot guarantee the authenticity of the sampling and result reporting. Moreover, several antigen test strips from different brands in the market have varying accuracy.

Based on this, the current focus should be developing an accurate, fast, easy-to-use self-testing method (Rader et al., 2022). Self-test (home test, at-home test, or over-the-counter test) data include antigen and nucleic acid amplification test (NAAT) results (Ritchey et al., 2022). In this study, we evaluated a nucleic acid self-testing device for detecting SARS-CoV-2 called GeneClick, based on the isothermal multiple self-pairing amplification technology (IMSA) (Ding et al., 2014). Six primers (including four hybrid primers) specifically identify seven sites on the target gene, generating a multiplicity of specific nucleotide structures capable of self-pairing followed by cyclic amplification at 65 °C. IMSA is an improvement and supplement to the loop-mediated isothermal amplification (LAMP) (Kitajima et al., 2021). Its most essential feature is that the special nucleotide structure multiples that can self-pair and trigger circular amplification are generated during the isothermal nucleic acid amplification process. Enriching the special nucleotide structures increases the chance of subsequent cyclic amplification, which increases the amplification efficiency, ultimately improving its sensitivity, which makes IMSA superior to LAMP detection.

In this study, we constructed a nucleic acid self-test platform named GeneClick, which is based on the RT-IMSA approach and microfluidic chip technology. To evaluate the performance of the nucleic acid self-test platform (GeneClick), we conducted a multicenter rapid nucleic acid self-test of SARS-CoV-2 RNA at the Fengtai District Center for Disease Control and Prevention (CDC) in Beijing, China, Alar Hospital of the First Division of Xinjiang Production and Construction Corps, China, and Savannakhet Provincial Hospital in Laos. To the best of our knowledge, this is the first report of nucleic acid self-test in China.

2. Materials and methods

2.1. Modules and features of GeneClick

The GeneClick device (consisting of three modules, a sampling kit, a microfluidic chip-based disposable cartridge, and an amplification reader) was manufactured by Baicare Biotechnology, Co., Ltd., Beijing, China.

2.1.1. Sampling kit

The sampling kit includes a nasal swab and a sample extraction tube. After sampling with a swab, the user places the swab into the sample extraction tube containing a lysis and amplification buffer, and rotates it slowly 15 times to elute the secretions from the nasal swab.

2.1.2. Disposable cartridge

The disposable cartridge (Fig. 1A) consists of a reaction tank that holds the freeze-dried microspheres containing primers and enzymes, which amplify the SARS-CoV-2 target sequences (*ORF1ab*, *N* gene), internal control (IC) (human *RnaseP* gene) and positive control

(PC) (recombinant plasmid), which aim to monitor effectiveness of sampling and amplification reaction.

2.1.3. Amplification reader

The amplification reader (Fig. 1B) consists of a heating element that controls temperature to allow for the isothermal amplification (IMSA) of target genes in the reaction tank. It also contains the detection reagent, Cresyl red, a pH indicator. During the amplification reaction, the by-products produced include pyrophosphate and hydrogen ions. If the sample tested is positive, the Cresyl red pH changes from alkaline (red) to acidic (yellow) as the reaction proceeds. However, if the sample tested is negative for SARS-CoV-2, the Cresyl red color (red) does not change. The amplification reader also contains a photodiode, which detects the light transmission intensity of Cresyl red through embedded software and displays the results through an indicator light following the detection.

2.1.4. Workflow

GeneClick has three distinctive features: real-time sampling monitoring, automatic results reporting, and rapid detection. A smartphone is needed to set up the sampling artificial intelligence monitoring during testing. First, the user needs to open the supporting application on the smartphone and scan the quick response (QR) code on the reagent. Next, the detection process is captured using the smartphone camera based on the testing steps as per the prompts on the phone. After completing the detection/amplification process, the test results are uploaded to the data center, where the test results are managed in a unified manner. The testing steps are simple, similar to the antigen self-test at home. Precisely, the nasal swab is placed into the nucleic acid release agent, which is then transferred into the sample tank in the microfluidic chip. The transferred liquid fills eight independent reaction pools after several seconds. The eight channels are set up in the chip, of which three are used to detect the *N* gene, three to detect the *ORF1ab* gene, a positive control, and an internal control to ensure the validity of the test, equivalent to repeating the test three times at the same time to improve the reliability of the test.

2.2. Detection principles

The GeneClick detection adopts the reverse transcription IMSA technology. Six specific primers were designed for seven target sequence regions, including four hybrid primers (DsF, DsR, FIT, and RIT) and two non-hybrid primers (SteR and SteF). An amplification process catalyzed by Bst DNA polymerase using DsF, DsR, FIT, and RIT primer sequences under isothermal conditions (around 65 °C) yields four original self-pairing structures. During the subsequent cyclic amplifications, the four hybrid primers continuously generate multiple self-pairing structures based on the four original self-pairing structures, determining the sensitivity and specificity of the amplification reaction. At the same time, the amplification reaction is significantly accelerated by adding the two non-hybrid primers. Fig. 2A shows the primer set of IMSA. Fig. 2B shows the generation of four original self-matching structures (SMS). The primer sequences used are shown in Supplementary Table S1.
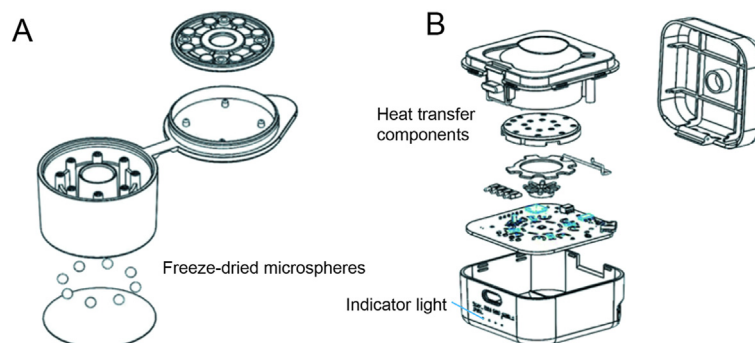

Fig. 1. Schematic diagram of disposable cartridge and amplification reader structure. **A** Disposable cartridge. **B** Amplification reader.
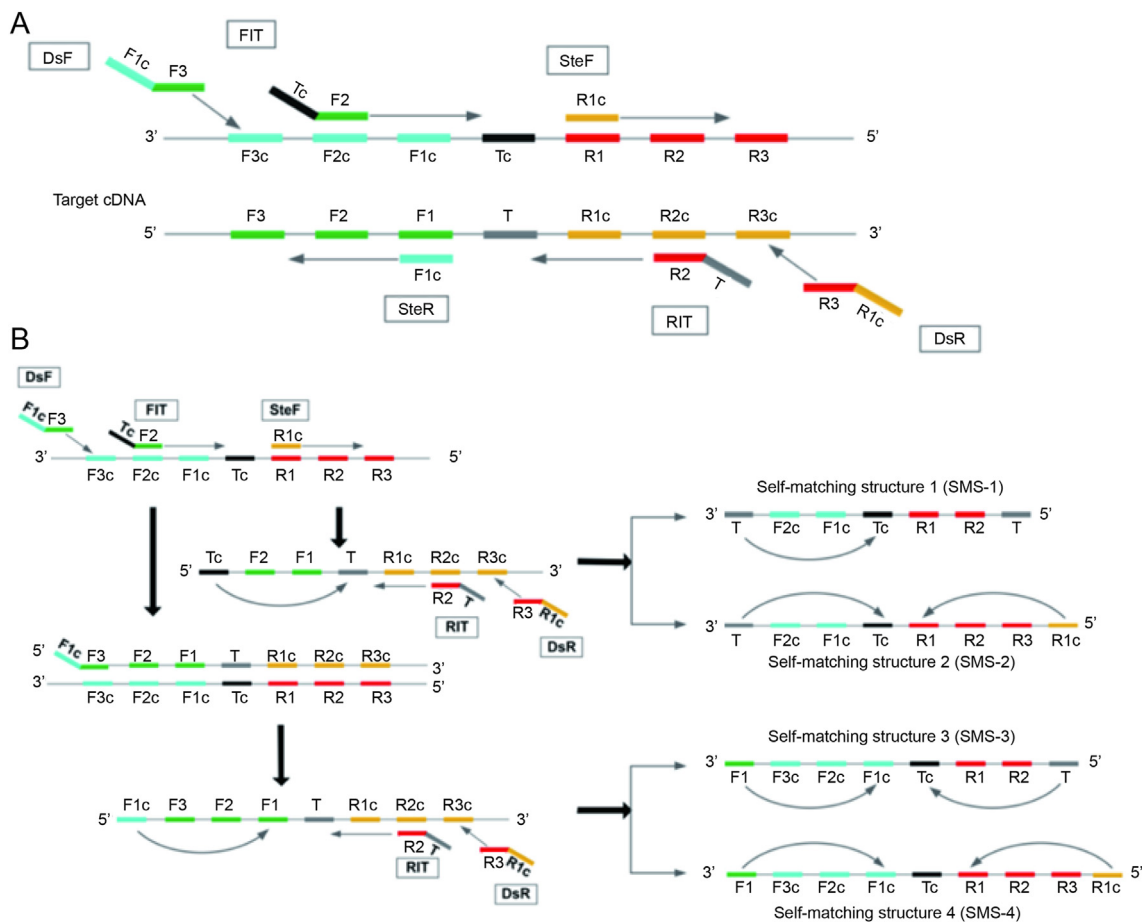
Fig. 2. The principle of IMSA amplification. **A** Primer design of RT-IMSA reaction. Six primers are used in the RT-IMSA assay, including two stem primers (SteF and SteR) and two pairs of nested hybrid-primers (two outer primers of DsF and DsR and two inner primers of FIT and RIT). The primers specifically recognize seven distinct regions of the target cDNA labeled F3, F2, F1, T, R1c, R2c, and *R3c* from the 5′ end. The DsF and DsR primers consist of the F3 and R3 and F1c and R1c sequences, the FIT and RIT primers consist of F2 and R2 and Tc and T sequences, and the SteF and SteR primers are the R1c and F1c sequences, respectively. **B** The initial step of RT-IMSA. For ease of explanation, DNA synthesis initiated from DsF, and FIT is set as the starting process (DNA synthesis proceeds with DsR and BIT in a similar manner). Horizontal straight lines with arrows represent the direction of primer elongation. Angled lines with arrows represent primers annealing to sites on the target. Arcs with arrows represent the self-matching function of two regions. In the step, four basic self-matching structures (SMS-1 to -4) with different lengths are generated.

2.3. Initial sensitivity and specificity assessment of GeneClick assay

Inactivated viral cultures of SARS-CoV-2 Wuhan strain for sensitivity assessment were obtained from the National Institute for Food and Drug Control. A digital PCR machine quantified the inactivated viral cultures. An initial concentration of 3×10^5 copies/mL of the inactivated viral cultures was then added to a negative matrix (negative nasal swab preservation solution) based on a 1:3 dilution gradient. Samples A–D were prepared as follows (samples A, B, C, and D corresponding to a final concentration of 1.23×10^3, 4.11×10^2, 1.37×10^2 and 0 copies/mL (negative control). Each sample was tested 20 times, with simultaneous comparisons with the commercially available RT-qPCR reagents (Sansure, Hunan, China). The viral nucleic acids used for specificity assessment were obtained from viral stock strains collected in our laboratory, including influenza A viruses (H1N1, H3N2), influenza B viruses (BV, BY), human parainfluenza viruses, respiratory syncytial viruses, and adenovirus types 3 and 7 preserved at the National Institute of Viral Disease Prevention and Control. We also examined the detection feasibility of GeneClick assay for the COVID-19 Omicron mutants (BF7 and BA5 strains prevalent in China) by analyzing the sequence homology *in Silico*. All the assessments were conducted in duplicate. All the testing was completed at the Institute of Viral Disease Prevention and Control, Chinese Center for Disease Control and Prevention. Information on the

strains used for the specificity assessment of the GeneClick experiment is shown in Table 1.

2.4. Assessment of the SARS-CoV-2 infection detection using GeneClick assay

2.4.1. Source of the samples

A total of 2162 samples were tested for SARS-CoV-2 infection using GeneClick assay between July and December 2022. All samples were nasal swabs, of which 41 were collected and tested at the Fengtai District CDC and Prevention, 45 at Alar Hospital of the First Division of Xinjiang Production and Construction Corps, and 2076 at the Savannakhet Provincial Hospital in Laos. All the protocols in this study were reviewed and approved by the Institutional Review Boards of local CDCs and the hospital mentioned above in accordance with the national ethics regulations.

2.4.2. GeneClick assay

Samples were self-collected using a nasal swab (provided with the sampling kit) by rotating five times around the internal wall of both nostrils (Fig. 3). The collected nasal swabs were independently placed into the sample extraction tube (provided with the sampling kit) and slowly rotated 15 times before squeezing all the liquid into the sampling tube. The liquid in the sampling tube was then poured into the reaction

Table 1
Information on the strains used for the specificity assessment of the GeneClick assay.

| Virus	Origin	Sample types	GeneClick testing results
Influenza A (H1N1, H3N2)	Jiangsu CDC (Jiangsu, China)	Pharyngeal swabs	Negative
Influenza B (Victoria Lineage, Yamagata Lineage)	Jiangsu CDC (Jiangsu, China)	Pharyngeal swabs	Negative
Human respiratory syncytial viruses	Beijing Children's Hospital	Pharyngeal swabs	Negative
Human parainfluenza virus	ATCC VR-94	Nasopharyngeal aspirates	Negative
Human adenovirus 3/7	Hebei CDC (Hebei, China)	Pharyngeal swabs	Negative
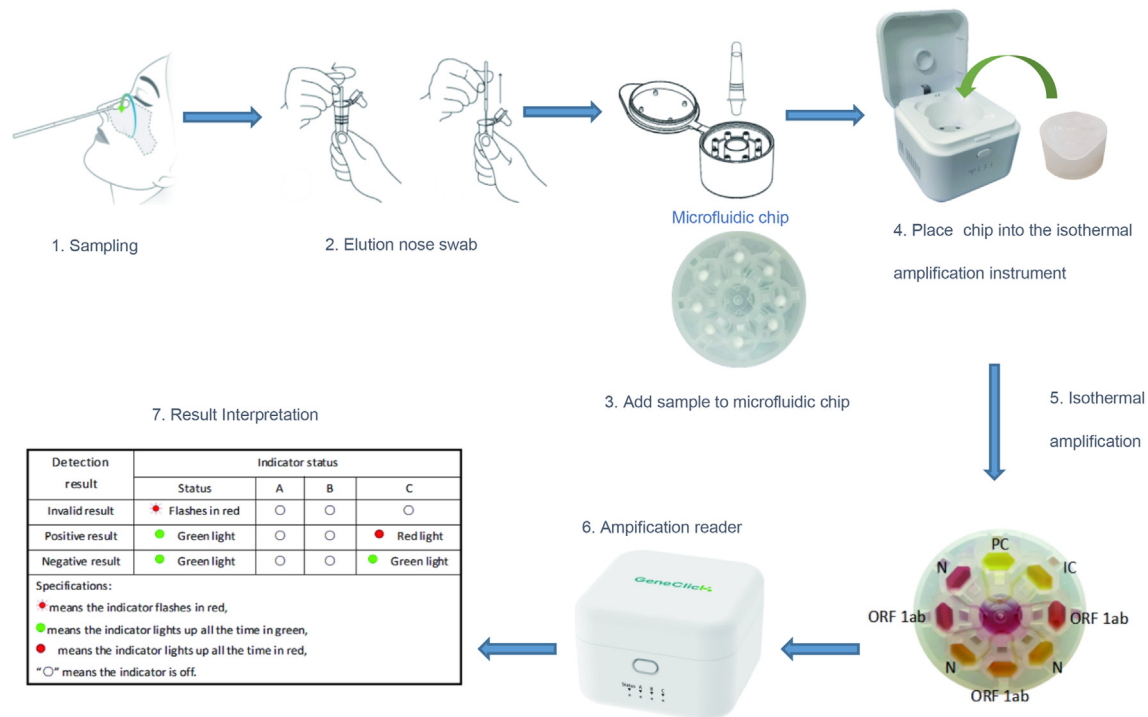
Fig. 3. Schematic diagram of the testing process. (1) Roll the swab along the wall of the nasal cavity for at least five times using the swab in the sampling kit. (2) Immerse the swab into the sample extraction tube and rotate it slowly for 15 times to elute the secretions from the nasal swab. (3) Squeeze all the liquid in the sampling tube into the hole in the center of the disposable cartridge at once. (4) Open the top cover of the instrument, and place the test device with the sample into the detection cartridge of the instrument in the right direction. The instrument runs automatically after closing its top cover. The status indicator light will turn green when the test is running. (5) Perform isothermal amplification reaction at 65 °C for 40 min. (6) The instrument detects the light transmission intensity of liquid with a photodiode, judges the algorithm through embedded software, and displays the results through the indicator light after the detection. (7) Interpretation of test results.

tank in the test chip (the disposable cartridge); the lid of the test chip was closed and then placed into the isothermal amplification instrument (the amplification reader) until it produced a "clicked" sound. A "Status" light bulb should start blinking when the test runs. Testing was completed after 40 min, and the results were interpreted according to the instructions on the sampling kit.

2.4.3. Reference methods and analysis of discrepant results

The commercial RT-qPCR kits were used as reference methods/kits for detecting SARS-CoV-2. The 2162 samples were also tested using three different RT-qPCR kits, including Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit (fluorescence PCR method) (BioPerfectus technologies, Jiangsu, China), Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) (Sansure, Hunan, China), and Novel Coronavirus Nucleic Acid Detection Kit (RT-qPCR fluorescence probe method) (Biogerm, Shanghai, China). In addition, 2076 out of the 2162 samples were tested using the antigen rapid test kit from TopstroMed Medical Technology Co., Ltd., Zhejiang, China. All RT-qPCR and rapid antigen test kits were approved by National Medical Products Administration (NMPA) and certified by European Union. The parameters for the RT-qPCR kits are shown in Supplementary Table S2.

2.5. Statistical analysis

The data obtained by different assays were analyzed using IBM SPSS Statistics 26 (IBM Corporation, NY, USA). Kappa values (κ) were used to measure the agreement between GeneClick and RT-qPCR/antigen testing results.

3. Results

3.1. The sensitivity and specificity of GeneClick assay

To evaluate the sensitivity of GeneClick, a series of diluted inactivated viral culture of SARS-CoV-2 were adopted: samples A, B, C and D were made corresponding to final concentrations of 1.23×10^3, 4.11×10^2, 1.37×10^2 and 0 copies/ml (negative control), respectively. Each sample was tested for 20 repetitions. The results of sample A using GeneClick were consistent with those of RT-qPCR, and sample B had one test not identified by GeneClick. Sample C using RT-qPCR had 14 positives and 6 negatives. GeneClick, on the other hand, tested 9 positives and 11 negatives, with an overall compliance rate of 75%. Sample D was a negative substrate and tested negative using both assays. Comparative test results are shown in Table 2. The limit of detection (LOD) of the GeneClick assay

Table 2

Sensitivity test results of GeneClick and RT-PCR.

Sample A			Sample B			Sample C			Sample D		
RT-PCR (Ct value)		GeneClick	RT-PCR (Ct value)		GeneClick	RT-PCR (Ct value)		GeneClick	RT-PCR (Ct value)		GeneClick
N	*ORF1ab*		*N*	*ORF1ab*		*N*	*ORF1ab*		*N*	*ORF1ab*	
36.88	34.76	Pos	38.98	37.08	Pos	36.33	38.16	Neg	U	U	Neg
35.44	34.27	Pos	39.70	36.76	Pos	U	U	Pos	U	U	Neg
36.28	34.02	Pos	38.81	36.50	Pos	37.25	36.94	Neg	U	U	Neg
36.00	34.22	Pos	38.41	36.16	Pos	36.96	37.85	Pos	U	U	Neg
36.22	34.45	Pos	38.86	36.78	Pos	37.68	U	Neg	U	U	Neg
35.59	34.37	Pos	36.84	35.95	Pos	38.41	37.95	Pos	U	U	Neg
37.25	34.74	Pos	37.71	37.49	Pos	36.84	37.93	Pos	U	U	Neg
35.92	34.90	Pos	37.48	37.32	Pos	37.71	37.18	Pos	U	U	Neg
35.66	34.25	Pos	38.05	35.63	Pos	36.67	37.86	Neg	U	U	Neg
35.86	34.75	Pos	36.94	36.40	Pos	39.10	37.63	Pos	U	U	Neg
36.37	34.73	Pos	37.96	36.26	Neg	36.80	39.38	Neg	U	U	Neg
36.29	34.27	Pos	36.80	37.46	Pos	37.40	U	Pos	U	U	Neg
37.29	33.97	Pos	37.99	36.19	Pos	36.82	37.48	Neg	U	U	Neg
36.34	34.93	Pos	36.93	36.05	Pos	39.57	U	Pos	U	U	Neg
36.85	34.49	Pos	37.32	37.60	Pos	37.08	37.32	Neg	U	U	Neg
35.78	34.71	Pos	38.25	37.08	Pos	U	U	Pos	U	U	Neg
35.97	35.14	Pos	37.82	36.34	Pos	39.92	39.46	Neg	U	U	Neg
36.41	34.70	Pos	36.88	36.41	Pos	37.39	U	Neg	U	U	Neg
36.44	34.35	Pos	37.11	36.63	Pos	37.91	38.11	Neg	U	U	Neg
36.81	34.10	Pos	38.30	37.39	Pos	37.85	37.08	Neg	U	U	Neg

Pos, positive; Neg, negative; U, undetected.

was 4.11×10^2 copies/mL. Compared to the RT-qPCR kits, the positive consistency rate was 95%, and the negative consistency rate was 100%, with an overall consistency rate of 95% (Table 3).

To assess the specificity of GeneClick, viral nucleic acids extracted from viral stock strains of influenza A viruses (H1N1, H3N2), influenza B viruses (BV, BY), human parainfluenza viruses, respiratory syncytial viruses, and adenovirus types 3 and 7 (Table 1) were used for GeneClick assay. No cross-reactivity was observed.

3.2. Clinical performance evaluation of GeneClick assay

Consistency evaluation using the different assays at three different medical institutions revealed that among the 41 nasal swabs collected at Fengtai District CDC and Prevention, the GeneClick assay detected 35 positives and 6 negatives, while the Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit (fluorescence PCR method) detected 36 positives and 5 negatives. The Ct values ranged from 16.23 to 37.04, and the kappa value was 0.895 ($P < 0.01$). Among the 45 samples collected from Alar Hospital of the First Division of Xinjiang Production and Construction Corps, the RT-qPCR assay detected 36 positives and 9 negatives, with Ct values between 22.17 and 39.89. On the contrary, the GeneClick assay detected 33 positives and 12 negatives with a kappa value of 0.815 ($P < 0.01$). The 2076 samples tested at the provincial hospital in Savannakhet Province, Laos, had Ct values ranging from 18.8 to 38.5. RT-qPCR detected 314 positives and 1762 negatives, while GeneClick detected 315 positives and 1761 negatives, with a kappa value of 0.983 ($P < 0.01$). Out of 2162 samples tested, 13 samples had discrepant results. GeneClick missed eight positive samples, while RT-qPCR missed five positive samples. This inconsistency may be attributed to sampling error, extremely low viral load (Ct values greater than 36.3), or miss operation (Table 4).

Table 3

Consistency of GeneClick and RT-PCR testing.

	Sample A	Sample B	Sample C	Sample D
Positive consistency rate (%)	100	95	64	100
Negative consistency rate (%)	100	100	100	100
Overall consistency rate (%)	100	95	75	100

Pos, positve; Neg, negative; U, undetected.

Among the 314 positive samples detected by GeneClick assay at the provincial hospital in Savannakhet Province, Laos, 63 (approximately 20%) were missed by the Novel Coronavirus (SARS-CoV-2) Antigen Rapid Test kit. These discrepant samples had Ct values ranging between 27.4 and 36.3. These results are shown in Table 5.

4. Discussion

SARS-CoV-2 is still a declared global pandemic. Therefore, a rapid and easy-to-use nucleic acid self-testing, particularly for home use or hospital emergency rooms, community hospitals, nursing homes, kindergartens, schools and universities, and other high-traffic areas, is highly desired to reduce the outbreak and transmission risks significantly (Crozier et al., 2021; Paltiel et al., 2022; Rubin, 2021). So far, the NMPA of China has authorized the use of 42 nucleic acid tests and 50 antigen test kits for the national detection of COVID-19. However, routine nucleic acid testing using RT-qPCR and RT-RAA kits require professionally trained laboratory personnel for nucleic acid extraction (Rai et al., 2021; Shen et al., 2021; Wang et al., 2020), while nucleic acid-based point-of-care-testing is not adequately designed for the self-testing (Ma, 2022).

Nucleic acid self-testing technology is developing rapidly. Currently, three molecular test kits, including Cue COVID-19 Test (Donato et al., 2021), Lucira Check-It COVID-19 Test (Zahavi et al., 2022), and Detect Covid-19 Test (Bruijns et al., 2022), have an Emergency Use Authorization by Food and Drug Administration for at-home use. These three kits are based on isothermal amplification methods using a nasal swab for self-sampling. However, the LOD of these kits is 800–1200 copies/mL, and the cost of consumed reagents per test is over $50 per person. In contrast, the GeneClick assay uses the IMSA method. In principle, IMSA technology generates four self-pairing structures during the detection reaction *vs* two self-pairing structures in the LAMP, resulting in a higher amplification efficiency than LAMP, contributing to the higher sensitivity in the GeneClick assay. The LOD in the GeneClick assay was 4.11×10^2 copies/mL, which is lower than the 500 copies/mL required by NMPA. In addition, a disposable cartridge (reaction regent) costs about $3, far less than that of the three at-home-use approved molecular test kits. In addition, the clinical performance of GeneClick assay based on the 2162 samples from the three medical institutions demonstrated that GeneClick is comparable to the conventional RT-qPCR kits used in the laboratories, with a kappa value of 0.98.

Table 4

Consistency analysis of RT-qPCR method and GeneClick assay for SARS-CoV-2 detection.

Group	GeneClick	RT-qPCR		Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa
		Pos	Neg						
A	Pos	35	0	35	97.22	100	100	83.33	0.895
	Neg	1	5	6					
	Total	36	5	41					
B	Pos	33	0	33	91.67	100	100	75	0.815
	Neg	3	9	12					
	Total	36	9	45					
C	Pos	310	5	315	98.73	99.72	98.41	99.77	0.983
	Neg	4	1757	1761					
	Total	314	1762	2076					
Total	Pos	378	5	383	97.93	99.72	98.69	99.55	0.979
	Neg	8	1771	1779					
	Total	386	1776	2162					

Group A: Fengtai District CDC and Prevention; group B: Alar Hospital of the First Division of Xinjiang Production and Construction Corps; group C: the Savannakhet Provincial Hospital in Laos.

Pos, positive; Neg, negative; PPV, positive predictive value; NPV, negative predictive value.

Calculating formula: sensitivity = true positive/(true positive + false negative); specificity = true negative/(true negative + false positive); PPV = true positive/(true positive + false positive); NPV = true negative/(false negative + true negative).

Table 5

Consistency analysis of antigen self-test and GeneClick assay for SARS-CoV-2 detection.

GeneClick	Ag-test		Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa
	Pos	Neg						
Pos	251	63	314	100	100	79.94	96.55	0.871
Neg	0	1762	1762					
Total	251	1825	2076					

Ag-test, antigen-test; Pos, positive; Neg: negative; PPV, positive predictive value; NPV, negative predictive value.

Calculating formula: sensitivity = true positive/(true positive + false negative); specificity = true negative/(true negative + false positive); PPV = true positive/(true positive + false positive); NPV = true negative/(false negative + true negative).

Currently, the mainly used self-testing kit is the rapid antigen test kit for home use. However, it is limited by its detection principle and low detection sensitivity. The antigen self-testing can steadily detect a minimum viral load of 10^5–10^6 copies/mL (or Ct value < 25) (Cubas-Atienzar et al., 2021), increasing the chances of transmission by asymptomatic persons. However, this study demonstrated that GeneClick is superior to antigen self-testing at home. GeneClick identified 63 samples undetected by the antigen test, indicating the higher sensitivity of GeneClick compared to antigen-based detection. Besides, GeneClick validates the collected samples using a smartphone through artificial intelligent monitoring. The test results are also automatically uploaded in real-time, enabling accurate location tracking, data sharing, and rapid detection while promoting a multi-scene intelligent warning. At the same time, the reaction kit (disposable cartridge) uses reagent lyophilization technology to ensure that the product can be stored and transported at room temperature. Furthermore, the entire amplification process occurs in a closed chip, effectively protecting against biosafety risks. Though GeneClick is more costly than antigen test strips, the cost of GeneClick can be reduced further by scaling up or performing mixed nucleic acid tests in the family.

GeneClick may also easily expand to simultaneously detect multiple pathogens since it has eight independent channels in the disposable cartridge, such as respiratory diseases highly prevalent during winter (Moriyama et al., 2020). In recent years, other than SARS-CoV-2, influenza A virus, adenovirus, and respiratory syncytial virus outbreaks have also caused severe public health problems (Chung et al., 2021; Wu et al., 2020). Given the many pathogens causing respiratory infections, patients may carry more than one pathogen. The overlap of clinical signs and symptoms caused by the different pathogens, including viruses and bacteria, often makes the etiological diagnosis difficult based on clinical presentation alone (Li et al., 2020). Subsequently, failure to identify the cause of the disease results in failure of the conventional treatment, antibiotic overuse, and cross-infection. The COVID-19 infection can become chronic, like influenza, with long-term and substantial effects and damages (Telenti et al., 2021). Therefore, future research should focus on developing GeneClick-based differential diagnoses of COVID-19 and common infections such as pneumonia for timely isolation of COVID-19 patients to prevent the spread of COVID-19.

This study had some limitations. First, sample collection was conducted by laboratory staff who performed the GeneClick tests in a point of care (POC) environment; thus, the home self-test aspect was not fully realized. However, several adult studies have evaluated using self-collected samples for SARS-CoV-2 molecular testing and have demonstrated 93%–97% concordance rates with standard swabs obtained by healthcare workers (Youngster, 2022). Secondly, the GeneClick assay can only detect one sample at a time. Thirdly, the GeneClick assay cannot accurately quantify the number of virus copies.

5. Conclusions

GeneClick is more accurate than the antigen detection kits and perfectly adapts to all scenarios where antigen detection is applicable. With the additional features of simple operation, affordable price, portable devices, and reliability of smartphone APP-driven sampling and result reporting, GeneClick offers a powerful tool for SARS-CoV-2 detection in primary healthcare institutions or at-home use.

Data availability

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

All aspects of the study were performed in accordance with national ethics regulations and approved by the Institutional Review Boards of

local Centers for Disease Control (CDCs) and hospital mentioned above. Informed consent was obtained from each participant.

Author contributions

Conflict of interest

Zhang Guohao reported employment from Beijing Baicare Biotechnology, Co., Ltd. The company provided the GeneClick instrument and reagents with the field experiments. The company had no role in the preparation, review, or approval of the manuscript and decision to submit the manuscript for publication. No other disclosures were reported.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.06.009.

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