Clin. Lab. 2025;71:XXX-XXX

ORIGINAL ARTICLE

Clinical Usefulness of the Loop-Mediated Isothermal Amplification Assay for SARS-CoV-2 Detection

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SUMMARY

Background: **Loop-mediated isothermal amplification (LAMP) is a molecular diagnostic method known for its rapid processing and operational simplicity due to its isothermal amplification process. While LAMP has demonstrated comparable diagnostic accuracy to PCR in certain applications, its performance may vary depending on assay design and implementation. This study aimed to evaluate the diagnostic performance of the MmaxSure™ assay (MmaxSure™; Mmonitor, Daegu, South Korea) in detecting SARS-CoV-2, comparing it with the STANDARD™ M nCoV Real-Time Detection Kit (STANDARD; SD BioSensor, Suwon, South Korea) using nasopharyngeal and oropharyngeal swab specimens.**

Methods: **A total of 333 specimens were included in the analysis, consisting of 113 positive and 220 negative nasopharyngeal and oropharyngeal swab samples. All specimens were tested using the MmaxSure™ assay, and the results were compared to those obtained using the STANDARD™ M nCoV Real-Time Detection Kit. The diagnostic performance of the MmaxSure™ assay was evaluated in terms of positive percent agreement (PPA), negative percent agreement (NPA), and Cohen's kappa index for inter-assay agreement. Sensitivity, specificity, and limit of detection (LOD) for SARS-CoV-2 variants were also determined.**

Results: **The MmaxSure™ assay demonstrated a 100% PPA and a 100% NPA with the STANDARD™ M nCoV Real-Time Detection Kit. The Cohen's kappa index was 1.0, indicating perfect agreement between the two diagnostic methods. The MmaxSure™ assay exhibited a high sensitivity, detecting SARS-CoV-2 variants at a LOD of 2 - 4 copies/μL, without cross-reactivity with other pathogens.**

Conclusions: **The MmaxSure™ Fast SARS-CoV-2 Detection Kit, based on LAMP technology, exhibited a high level of diagnostic accuracy in detecting SARS-CoV-2. Its rapid turnaround time and minimal equipment requirements suggest its potential suitability for point-of-care applications. However, further prospective studies with a broader range of clinical specimens and real-world validation are needed to confirm its diagnostic utility across diverse settings.**

(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2025.250327)

Manuscript accepted April 30, 2025

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KEYWORDS

LAMP assay, diagnostic performance, molecular diagnostics, SARS-CoV-2

INTRODUCTION

Loop-mediated isothermal amplification (LAMP) is a molecular diagnostic method that enables rapid nucleic acid amplification under isothermal conditions. Its simplified amplification process allows for faster detection compared to conventional PCR methods, typically requiring less than half the time [1,2]. Owing to these advantages, LAMP technology is being applied to detection kits for various pathogens, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3-5].

The MmaxSure™ Fast SARS-CoV-2 Detection Kit (MmaxSure™; Mmonitor, Daegu, South Korea), which employs LAMP technology, was approved by the Ministry of Food and Drug Safety in Korea on March 29, 2023. It was designed to provide a rapid and accessible alternative to conventional RT-PCR by enabling the amplification and detection of SARS-CoV-2 through a simple visual color change of a fluorescent dye, eliminating the need for specialized PCR and detection equipment. However, as with other LAMP-based assays, it still requires external RNA extraction before amplification. Future advancements in integrated RNA extraction and amplification techniques may further enhance the applicability of LAMP-based assays in point-of-care settings.

This study aimed to compare the diagnostic performance of the MmaxSure™ assay with that of the STANDARD™ M nCoV Real-Time Detection Kit (STANDARD; SD BioSensor; Suwon, South Korea), which is commonly used to detect SARS-CoV-2 infections in hospital.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Kyungpook National University Chilgok Hospital, Daegu, Korea (approval number: KNUCH 2022-04-032). Informed consent was waived for all subjects, because patient information was anonymized and de-identified prior to the retrospective analysis using residual specimens scheduled for disposal. All experiments were performed in accordance with relevant guidelines and regulations.

Nasopharyngeal and oropharyngeal swab specimens were collected from patients suspected of SARS-CoV-2 infection at Kyungpook National University Chilgok Hospital between March 2022 and April 2022. The swab specimens were stored in viral transport medium (VTM; Noble Bio, Hwaseong, South Korea) and subjected to routine SARS-CoV-2 testing using the STANDARD assay. Leftover specimens with more than 200 µL, the minimum amount required for RNA extraction, were stored at -70℃ for use in this study.

A total of 333 specimens (113 SARS-CoV-2-positive and 220 SARS-CoV-2-negative) were tested using the MmaxSure™ assay. RNA extraction was performed using the QIAamp® DSP Viral RNA Mini Kit (Qiagen, Hilden, Germany). Positive specimens were selected based on the cycle threshold (Ct) values obtained from routine testing using the STANDARD assay.

The MmaxSure™ assay was performed following the manufacturer's instructions. Briefly, 5 µL of RNA extraction from the specimen was mixed with 20 µL of MmaxSure™ LAMP premix in a 200 µL PCR tube. The mixture was incubated at 58℃ for 30 minutes and then heated at 80℃ for 2 minutes to terminate the reaction. A specimen was considered positive for SARS-CoV-2 if a color change occurred from purple (HEX color code: #744F8F) to blue (#416B97) in both the RdRp and N tubes (Supplemental Data Figure S1) [6].

Due to proprietary concerns, the specific sequences of the primers used in the MmaxSure™ assay cannot be disclosed. However, the assay targets highly conserved regions of the SARS-CoV-2 genome, specifically the RdRp and N gene regions, which are known to provide reliable and accurate detection of the virus. The primer sequences are optimized for use in LAMP-based amplification to ensure high sensitivity and specificity.

For nasopharyngeal and oropharyngeal swab specimens, which were confirmed positive or negative using the STANDARD assay, the correlation evaluation with the approved product was compared against the hospital's confirmed results.

RESULTS

As shown in Table 1, the positive percent agreement (PPA) was 100.0% (113/113 samples), and the negative percent agreement (NPA) was 100.0% (220/220 samples). The overall agreement, encompassing both positive and negative agreements, was 100.0% (333/333 samples). The lower limit of the 95% confidence interval for PPA was ≥ 96.8%. The lower limit of the 95% confidence interval for NPA was ≥ 98.3%. The Cohen's kappa index for overall agreement was 1.0, exceeding the evaluation standard of 0.8, indicating perfect agree-

Table 1. Clinical performance of the MmaxSure™ Fast SARS-CoV-2 Detection Kit compared to the STANDARD™ M nCoV Real-Time Detection Kit.

SARS-CoV-2 Diagnosis			STANDARD™ M nCoV Real-Time Detection Kit		
			positive	negative	total
MmaxSure™ Fast SARS-CoV-2 Detection Kit	positive		113 (33.93%)	0 (0.00%)	113 (33.93%)
	negative		0 (0.00%)	220 (66.07%)	220 (66.07%)
	total		113 (33.93%)	220 (66.07%)	333 (100.00%)
	sensitivity (%)	[95% CI] *	[99.6 to 100.0]		
		[95% CI] †	[97.8 to 100.0]		
		[95% CI] ‡	[96.8 to 100.0]		
	specificity (%)	[95% CI] *		[99.8 to 100.0]	
		[95% CI] †		[98.9 to 100.0]	
		[95% CI] ‡		[98.3 to 100.0]	
	Cohen's kappa index (%)	[95% CI] *			1.0 [1.0 to 1.0]
		[95% CI] †			
		[95% CI] ‡			

Data are displayed as the number of samples (percentage of incurred samples).
*** Confidence interval calculated using the Wald's method with continuity correction.**
† Confidence interval calculated using the Jeffreys-Perks method.
‡ Confidence interval calculated using the Clopper-Pearson method.
CI - confidence interval.

Table 2. Analytical sensitivity evaluation of the MmaxSure™ Fast SARS-CoV-2 Detection Kit.

RdRp gene	Concentration (copies/µL)	N gene
	positives/replicates	positives/replicates
100	120/120	120/120
10	120/120	120/120
4	120/120	120/120
2	12/120	10/120
0.2	0/120	0/120

Data are combined from multiple experiments.
RdRp - RNA-dependent RNA polymerase, N - nucleocapsid protein.

ment between the two assays.

To validate the positive results of the MmaxSure™ assay, the distribution of Ct values in the positive samples was identified by real-time PCR (Figure 1). The median Ct values for the RdRP and E genes were 15.34 and 15.2, respectively. The percentage of the MmaxSure™ positive group with Ct values > 30 was 14.16% (16/113), and the Ct values of all samples were lower than 35.

The limits of detection (LoD) for the MmaxSure™ assay targeting the SARS-CoV-2 RdRp and N genes were determined through serial dilutions of synthetic RNA corresponding to each gene. For the RdRp gene, the LoD was found to be between 2 - 4 copies/µL, respectively (Table 2). These findings indicate that the assay can reliably detect very low concentrations of SARS-CoV-2 RNA in a sample. LoD determination was performed in triplicate for each dilution, and the lowest concentration that resulted in successful amplification in 95% of the replicates was considered the LoD. The analytical specificity of the MmaxSure™ assay was evaluated by testing a panel of respiratory pathogens, including common viruses such as influenza A, influenza B, and Rhinovirus, as well as bacterial pathogens that could be present in similar clinical specimens. The assay showed no cross-reactivity with these respiratory

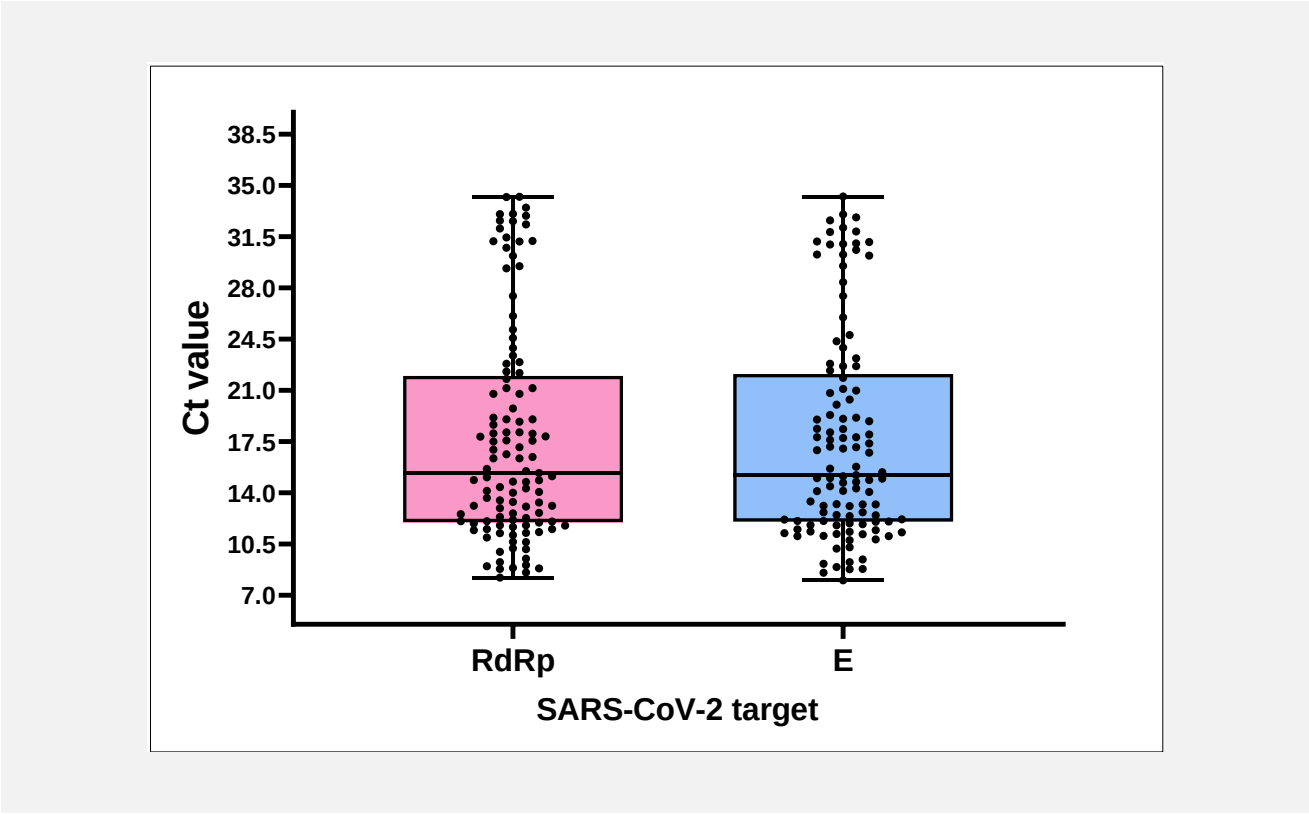

Figure 1. Distribution of Ct values of real-time PCR according to the positive results of the MmaxSure™ assay.

This box-and-whisker plot illustrates the distribution of Ct values of MmaxSure™ assay-positive specimens selected for this study. The bottoms and tops of the boxes represent the 25th and 75th percentiles, respectively. The horizontal lines in the boxes represent the median values. Ct - cycle threshold, RdRp - RNA-dependent RNA polymerase, E - envelope.

pathogens, except for SARS-coronavirus-1 (Supplemental Data Table S1). This confirms the high specificity of the assay for detecting SARS-CoV-2. Furthermore, the inclusivity of the MmaxSure assay was tested by analyzing several SARS-CoV-2 variants of concern, including the Alpha, Delta, and Omicron variants. The assay successfully detected all these variants, with a LoD of 4 copies/μL for each variant (Supplemental Data Table S2), demonstrating the assay's broad applicability across different circulating SARS-CoV-2 strains.

DISCUSSION

Until recently, most SARS-CoV-2 molecular diagnostic tests involved extracting RNA and then using RT-PCR to identify the genes of SARS-CoV-2 [7,8]. Conventional RT-PCR requires skilled personnel in molecular biology, specialized equipment, a significant amount of time, and high-cost reagents stored at -20°C. To address these limitations, we developed the MmaxSure™ Fast SARS-CoV-2 Detection Kit (Mmonitor), a molecular diagnostic product designed for qualitative analysis using LAMP to detect the RdRp and N genes of SARS-CoV-2.

In our study, we confirmed that the LoD of the MmaxSure™ assay is lower than that of the RT-PCR method under our experimental conditions [9,10]. However, this does not necessarily indicate superior sensitivity in clinical practice, as factors such as sample quality, viral load distribution, and real-world variability must be considered. While the MmaxSure™ assay enables faster detection, further studies are required to validate its performance across a broader range of clinical conditions. The MmaxSure™ assay is user-friendly, requiring only the addition of samples to a reagent mix after RNA extraction. However, this reliance on external RNA extraction poses a limitation in point-of-care scenarios, as it necessitates additional equipment and processing steps. To overcome this challenge, future advancements should focus on integrating rapid RNA extraction techniques with LAMP-based detection systems to enable true point-of-care applicability. Additionally, since the results depend on colorimetric detection, there is a risk of misinterpretation due to inter-individual variability. To mitigate this issue, future research should focus on developing a quantitative method for measuring color changes, potentially using a portable colorimeter or

smartphone-based image analysis.

To compare the MmaxSure™ assay with conventional RT-PCR, we used the same RNA extraction method for both assays. However, to fully leverage the advantages of LAMP for point-of-care testing, RNA extraction and amplification should ideally be simplified or integrated into a single step. In a previous study, we demonstrated nucleic acid amplification using a hot pack and detection on a lateral flow assay strip without specialized equipment [11]. Methods for extracting RNA without specialized equipment have been developed in the field of lab-on-a-chip, such as electrophoretic purification, hybridization-based purification using conjugated beads, and silica-based solid phase extraction [12,13]. After testing various RNA extraction methods, we developed a silica-based paper-chip tip as a laboratory-scale prototype, which demonstrated the highest RNA binding affinity and confirmed that the RNA yield rate was comparable to that of conventional methods. With all these advancements, the WHO ASSURED criteria for point-of-care tests could be satisfied [7].

To further validate the performance of the MmaxSure™ assay, future studies should include a larger sample size and diverse specimen types beyond nasopharyngeal and oropharyngeal swabs. Additionally, long-term stability studies under various environmental conditions should be conducted to ensure the robustness of the assay in different settings.

One of the main limitations of the MmaxSure™ assay is that it is a qualitative test, meaning it can only determine the presence or absence of SARS-CoV-2 RNA, without providing information on viral load. This poses a significant challenge in clinical settings where viral load is a critical factor, such as in monitoring disease progression or evaluating the effectiveness of treatments. The inability to quantify viral load may limit the accuracy of clinical decision-making, especially in cases with low viral loads, where understanding the amount of virus is crucial for tailoring treatment strategies. To enhance the clinical utility of the MmaxSure™ assay, further studies should explore approaches to address this limitation, particularly by integrating quantitative viral load data into LAMP assays, which would represent a key advancement in its diagnostic capabilities.

This study has several limitations. The sample set included only 14.16% of cases with Ct values > 30, with the majority having Ct values < 20, potentially leading to an overestimation of sensitivity. Additionally, due to the retrospective nature of this study, specimens near the cutoff value were not included, which limited our ability to assess the assay's performance at the critical threshold. This is an important consideration for future studies to evaluate how the MmaxSure™ assay performs at or near the cutoff, where sensitivity and specificity become more crucial. Furthermore, the assays were not performed in parallel; the STANDARD assay was conducted at the time of initial clinical evaluation, whereas the MmaxSure™ assay was later performed using leftover specimens. Given that the MmaxSure™ assay provides only qualitative results and that the STANDARD assay had already been clinically validated, repeating the STANDARD assay at the time of MmaxSure™ testing would not have significantly altered the interpretation of our findings.

In this study, the MmaxSure™ assay using LAMP demonstrated a lower LoD than the comparator RT-PCR assay under experimental conditions. While our findings suggest the potential clinical utility of the MmaxSure™ assay for rapid SARS-CoV-2 detection, further prospective studies are crucial to establish its diagnostic accuracy and performance across diverse populations and real-world settings.

Source of Funds:

This work was supported by the Korea Medical Device Development Fund grant funded by the Korean government (project number: HW20C2057) and a grant from the MD-PhD/Medical Scientist Training Program through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea.

Data Availability Statement:

All data generated during this study are available from the corresponding author upon request.

Declaration of Interest:

All authors report no conflicts of interest relevant to this article.

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