Brief Communication
Clinical Microbiology

Ann Lab Med 2023;43:508-511
https://doi.org/10.3343/alm.2023.43.5.508
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Evaluation of the AdvanSure One-Stop COVID-19 Plus Kit for SARS-CoV-2 Detection Using a Streamlined RNA Extraction Method

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Real-time reverse transcription (rRT)-PCR, which is the reference standard for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, generally involves a time-consuming and costly RNA extraction step prior to amplification. We evaluated the performance of the AdvanSure One-Stop COVID-19 Plus Kit (LG Chem, Seoul, Korea), a novel rRT-PCR assay that can detect SARS-CoV-2 within 90 minutes using a streamlined RNA extraction method. In total, 509 nasopharyngeal swab (NPS) specimens (SARS-CoV-2 positive: N=205; SARS-CoV-2 negative: N=304) previously tested using the PowerChek SARS-CoV-2 Real-time PCR Kit (Kogene Biotech, Seoul, Korea) were tested using the AdvanSure assay. The limit of detection (LOD) of the AdvanSure assay was determined using serially diluted inactivated SARS-CoV-2. The positive and negative percent agreements between the AdvanSure and PowerChek assays were 99.5% (204/205) and 99.3% (302/304), respectively. The LODs of the AdvanSure assay for SARS-CoV-2 nucleocapsid and spike/RNA-dependent RNA polymerase genes were 672 and 846 copies/mL, respectively. The results show that the performance of the AdvanSure assay is comparable to that of the PowerChek assay used for routine SARS-CoV-2 testing, suggesting that the AdvanSure assay is a useful diagnostic tool for rapid and accurate detection of SARS-CoV-2 infection.

Key Words: AdvanSure, COVID-19, SARS-CoV-2, RNA extraction, Real-time reverse transcription PCR, Performance

Received: December 26, 2022
Revision received: February 13, 2023
Accepted: April 4, 2023

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Rapid and accurate diagnosis is necessary to contain the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and molecular assays based on real-time reverse transcription (rRT)-PCR are currently considered the reference standard for the diagnosis of SARS-CoV-2 infection [1, 2]. These assays generally require a time-consuming and costly RNA extraction step, which remains a major bottleneck in the diagnosis of SARS-CoV-2 infection [3, 4]. Furthermore, the unprecedented demand for SARS-CoV-2 molecular assays has led to a global shortage of RNA extraction kits [5]. One of the most effective approaches to overcome these challenges involves the development of molecular assays using a streamlined RNA extraction method [3-5].

The AdvanSure One-Stop COVID-19 Plus Kit (AdvanSure; LG Chem, Seoul, Korea), a novel rRT-PCR assay that uses a streamlined RNA extraction method, can detect SARS-CoV-2 in nasopharyngeal swab (NPS) specimens within 90 minutes and has recently received the Conformité Européenne in-vitro diagnostic marking. The AdvanSure assay is a single-tube multiplex assay

that detects the nucleocapsid (N) gene (FAM channel) and spike (S)/RNA-dependent RNA polymerase (RdRp) genes (VIC channel) of SARS-CoV-2. We compared the performance of the AdvanSure assay with that of the PowerChek SARS-CoV-2 Real-time PCR Kit (PowerChek; Kogene Biotech, Seoul, Korea) for detecting SARS-CoV-2 in NPS specimens. This study was approved by the Institutional Review Board of Samsung Medical Center, Seoul, Korea (approval number: 2022-03-096). The requirement for informed consent was waived because of the retrospective nature of the study and the use of anonymized patient data.

NPS specimens were collected from patients suspected of SARS-CoV-2 infection at Samsung Medical Center between September 2020 and February 2022. The specimens were stored in viral transport medium (VTM; Noble Bio, Hwaseong, Korea) and subjected to routine SARS-CoV-2 testing using the PowerChek assay. After routine testing, leftover specimens were stored at –70°C until use in this study. Five hundred and nine NPS specimens (205 SARS-CoV-2-positive and 304 SARS-CoV-2-negative specimens) were thawed and tested using the AdvanSure assay. Positive specimens were selected based on the cycle threshold (Ct) values obtained from routine testing using the PowerChek assay, which covered a wide range of Ct values (Supplemental Data Fig. S1).

The AdvanSure assay was performed according to the manufacturer's instructions. Briefly, 7.5 µL of NPS specimen was mixed with an equal volume of lysis buffer provided in the kit and placed in the CFX96 system (Bio-Rad, Hercules, CA, USA). The mixture was incubated at 37°C for 5 minutes, heated at 95°C for 5 minutes, and cooled at 4°C for 1 minute. Thereafter, 10 µL of premix and 5 µL of primer/probe mix were added to the mixture, resulting in a final reaction volume of 30 µL. The rRT-PCRs were run using the following cycling conditions: incubation at 50°C for 10 minutes and denaturation at 95°C for 5 minutes, followed by 40 cycles at 95°C for 10 seconds and 58°C for 30 seconds. A specimen was considered positive for SARS-CoV-2 if there was a sigmoidal amplification curve with a Ct value ≤40 in both the FAM (N gene) and VIC (S/RdRp genes) channels. A specimen was considered inconclusive and retested if there was a sigmoidal amplification curve with a Ct value ≤40 in only one of the two channels. If the result remained inconclusive after retesting, it was finalized as inconclusive. Discordant results between the AdvanSure and PowerChek assays were resolved using a third assay, Xpert Xpress SARS-CoV-2 (Xpert; Cepheid, Sunnyvale, CA, USA).

Positive percent agreement (PPA), negative percent agreement (NPA), and Cohen's kappa values were calculated to assess the level of agreement between the AdvanSure and PowerChek assays. For the calculation of agreement, inconclusive results from the AdvanSure assay were considered positive.

The analytical sensitivity of the AdvanSure assay was evaluated using AMPLIRUN TOTAL SARS-CoV-2 CONTROL (SWAB) (Vircell, Granada, Spain). This material was serially diluted in a pool of negative NPS specimens, and 20 replicates per dilution level were tested. The limit of detection (LOD) was determined using probit regression analysis. The analytical specificity of the AdvanSure assay was assessed by testing 28 strains of human respiratory pathogens (Supplemental Data Table S1). The strains were tested in triplicate at clinically relevant concentrations (bacteria or yeasts: $\geq 1\times10^6$ colony-forming units [CFU]/mL or copies/mL; viruses: $\geq 1\times10^5$ plaque-forming units [PFU]/mL, 50% tissue culture infectious dose [$TCID_{50}$]/mL, or copies/mL).

The PPA and NPA between the AdvanSure and PowerChek assays were ≥99.3% (Table 1). The Cohen's kappa value was 0.99, indicating a nearly perfect agreement between the two assays. Pearson's correlation analysis showed that the Ct values of the two assays were highly correlated (PowerChek envelope [E] and AdvanSure N genes: $R^2=0.9398$; PowerChek open reading frame 1ab [ORF1ab] and AdvanSure S/RdRp genes: $R^2=0.9456$; Supplemental Data Fig. S2). Five specimens showed discordant results but were confirmed to be positive for SARS-CoV-2 using the Xpert assay (Table 2). These specimens showed high Ct values ranging from 32.5 to 39.7 in the Xpert assay, indicating that the SARS-CoV-2 load was very low.

The LODs of the AdvanSure assay for the SARS-CoV-2 N and S/RdRp genes were 672 and 846 copies/mL, respectively (Table 3), which are slightly lower than those of the PowerChek assay claimed by the manufacturer (1,000 copies/mL for both the E and ORF1ab genes). In the analytical specificity test, the Ad-

Table 1. Clinical performance of the AdvanSure assay compared to that of the PowerChek assay

AdvanSure result	PowerChek result			PPA (95% CI)	NPA (95% CI)
	Positive	Negative	Total		
Positive	202	0	202	99.5 (97.3-100.0)	99.3 (97.6-99.9)
Inconclusive*	2	2	4		
Negative	1	302	303		
Total	205	304	509		

*For the calculation of agreement, inconclusive results from the AdvanSure assay were considered positive.
Abbreviations: PPA, positive percent agreement; NPA, negative percent agreement; CI, confidence interval.

Table 2. Details of five specimens showing discordant results between the AdvanSure and PowerChek assays

Specimen number	PowerChek assay			AdvanSure assay			Discrepancy resolution (Xpert assay)		
	Result	Ct value		Result	Ct value*		Result	Ct value	
		E	ORF1ab		N	S/RdRp		E	N
147	Positive	32.6	33.9	Inconclusive	0/37.0	37.0/0	Positive	32.5	35.4
349	Positive	34.4	34.1	Inconclusive	38.3/37.9	0/0	Positive	35.4	38.9
432	Positive	34.7	34.4	Negative	0	0	Positive	35.6	39.7
16	Negative	0	0	Inconclusive	37.3/38.9	0/0	Positive	35.2	38.9
400	Negative	0	0	Inconclusive	0/0	39.0/38.6	Positive	37.1	39.3

*Numbers before and after the slash indicate Ct values obtained from the initial test and retest, respectively.
Abbreviations: Ct, cycle threshold; E, envelope; ORF1ab, open reading frame 1ab; N, nucleocapsid; RdRp, RNA-dependent RNA polymerase.

Table 3. Analytical sensitivity evaluation results of the AdvanSure assay

Concentration (copies/mL)	N gene		S/RdRp genes	
	N positives/ N replicates	Mean Ct value	N positives/ N replicates	Mean Ct value
2,000	20/20	33.3	20/20	33.8
1,500	20/20	33.9	20/20	34.5
1,000	20/20	35.2	19/20	35.5
800	19/20	35.6	18/20	36.0
600	18/20	36.0	17/20	36.6
500	18/20	36.9	15/20	37.1
250	9/20	37.6	4/20	37.9
100	2/20	38.4	3/20	38.8
50	3/20	39.5	1/20	38.9
Probit LOD (copies/mL)	672 (95% CI: 574–833)		846 (95% CI: 733–1,026)	

Abbreviations: N, nucleocapsid; S, spike; RdRp, RNA-dependent RNA polymerase; LOD, limit of detection; CI, confidence interval; Ct, cycle threshold.

vanSure assay showed no cross-reactivity with other respiratory pathogens (Supplemental Data Table S1).

To date, more than 200 SARS-CoV-2 molecular assays have been granted emergency use authorization by the US Food and Drug Administration, the vast majority of which require viral RNA extraction from clinical specimens prior to amplification [6]. RNA extraction is crucial to remove potential inhibitors that may interfere with viral target gene amplification; however, conventional RNA extraction processes consisting of lysis and purification steps are time-consuming and costly [3, 4]. Recently, streamlined RNA extraction methods, such as treatment with proteinase K or heat treatment, have been suggested as alternatives to conventional RNA extraction methods [3, 5-18]. However, there are currently few commercial molecular assays available for detecting SARS-CoV-2 in clinical specimens using streamlined RNA extraction methods, and little is known about their performance [16]. According to our study findings, the AdvanSure assay using a streamlined RNA extraction method demonstrates analytical and clinical performance comparable to that of conventional rRT-PCR assays used for routine SARS-CoV-2 testing.

Most rRT-PCR assays for SARS-CoV-2 detection require automated nucleic acid extraction equipment and real-time thermocyclers and are suitable for moderate- to high-throughput batch testing [17]. They are high-complexity assays that require trained laboratory personnel and have turnaround times (TATs) of several hours. In contrast, specimen-to-result RT-PCR assays, such as Xpert Xpress SARS-CoV-2 and BioFire COVID-19 Test (BioFire Diagnostics, Salt Lake City, UT, USA), are low-complexity assays that can be used in point-of-care settings [6, 18]. In these assays, RNA extraction, amplification, and detection are performed in a closed system, minimizing cross-contamination. However, they have a relatively low throughput and are not suitable for high-volume laboratories. The major advantages of the AdvanSure assay are that it has a shorter TAT than rRT-PCR assays using conventional RNA extraction methods and higher throughput than specimen-to-result RT-PCR assays.

A major limitation of this study was that archived NPS specimens collected for routine SARS-CoV-2 were thawed and tested using the AdvanSure assay. Long-term storage and the freeze–thaw process may have induced the degradation of some viral RNA. Furthermore, the VTM used in this study did not contain a chaotropic agent to prevent RNA degradation. Although the AdvanSure assay showed a comparable clinical performance to routine SARS-CoV-2 testing, this limitation may have led to an underestimation of its clinical performance.

In conclusion, the performance of the AdvanSure assay using a streamlined RNA extraction method was comparable to that of the PowerChek assay. The COVID-19 pandemic has generated an unprecedented demand for molecular diagnostic test-

https://doi.org/10.3343/alm.2023.43.5.508

ing, and in this situation, the AdvanSure assay with a high-throughput capacity (up to 96 specimens per batch) and short TAT (90 minutes) can be a valuable diagnostic tool.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Conceptualization: Kim TY and Huh HJ; Data curation: Kim TY and Huh HJ; Methodology: Kim TY, Shim HJ, and Huh HJ; Validation: Kim TY and Huh HJ; Writing—original draft: Kim TY; Writing—review and editing: Jeong E, Kang M, Jang JH, Huh HJ, and Lee NY. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

This study was supported by Nano Material Technology Development Program (Nanoconnect Program) through the National Foundation of Korea (NRF) funded by Ministry of Science and ICT (No. NRF-2022M3H4A4097528). In addition, this study was supported by LG Chem, Seoul, Korea. The sponsors had no role in the study design, data collection and analysis, or manuscript writing.

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