









Comparison of the AdvanSure RV Plus Real-Time RT-PCR and Real-Q RV II Detection Assays for Respiratory Viruses

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Dear Editor,

Various multiplex molecular assays have been developed and need to be evaluated before use in clinical laboratories. We evaluated the analytical and diagnostic performances of two one-step real-time reverse-transcription PCR assays for detecting respiratory viruses: the AdvanSure RV-plus real-time PCR assay (AdvanSure; LG Chem, Seoul, Korea) and the Real-Q RV II Detection assay (Real-Q RV II; BioSewoom, Seoul, Korea). The AdvanSure assay can simultaneously detect 15 respiratory viruses, including influenza (INF) virus types A and B; respiratory syncytial virus types A and B; parainfluenza virus (PIV) types 1, 2, and 3; adenovirus (ADV); human metapneumovirus; human bocavirus; coronavirus (CoV) OC43, 229E, and NL63; human rhinovirus (HRV); and enterovirus (ETV) [1]. The Real-Q RV II Detection assay, a revised version of the Real-Q RV Detection assay (BioSewoom) [2, 3], can detect PIV 4 in addition to the viruses detectable by the AdvanSure assay; however, the Real-Q RV II cannot differentiate between CoV 229E and CoV OC43.

We compared the performances of the AdvanSure and Real-Q RV II assays for 336 clinical nasopharyngeal swab samples

collected from 316 patients with symptoms of respiratory tract infection between January 2016 and May 2018 at Samsung Medical Center, Seoul, Korea. After routine testing, residual samples were stored at -70°C for one to two years prior to analysis. The AdvanSure and Real-Q RV II assays were performed simultaneously. The Institutional Review Board of Samsung Medical Center approved the study (IRB No. SMC 2018-11-064) and waived the requirement for informed consent.

Nucleic acids were extracted from clinical samples using the AdvanSure E3 system (LG Chem) and Nextractor NX-48 (Geno-solution Inc., Seoul, Korea) for the AdvanSure and Real-Q RV II assays, respectively, and amplified using the respective assays (i.e. AdvanSure and Real-Q RV II assays), according to the manufacturers' instructions. Positive percent agreement (PPA), negative percent agreement (NPA), and kappa values were used to compare the results of the two assays. Samples with discordant results between the two assays were assessed via uniplex PCR and sequencing in a blind manner with primers identical to those of the AdvanSure and Real-Q RV II assays.

We evaluated the analytical sensitivity of both assays using commercially sourced viral nucleic acids for ADV, INF A, and

Received: August 18, 2020

Revision received: November 21, 2020

Accepted: March 15, 2021

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PIV 3 (AmpliRun DNA or RNA control; Vircell, Granada, Spain). Ten other microorganisms, including CoV HKU-1, dengue virus serotype 1, severe acute respiratory syndrome coronavirus 2, Zika virus, *Chlamydomydia pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, and *Streptococcus pneumoniae*, were used for analytical specificity evaluation.

Of the 336 samples, 275 (81.8%) and 264 (78.6%) were positive for viral infection by the AdvanSure and Real-Q RV II assays, respectively. The PPA, NPA, and kappa values are presented in Table 1. The PPA values for detection of all viruses were 83.3%–100%, except for ETV (50%). NPA values ranged from 97.5% to 100%. The kappa values ranged from 0.81 to 1.00, except for ETV (0.42).

Results were discordant for 39 samples. Among these, four samples showed discordant results for two viruses; thus, 43 discordant results were detected in total (Table 2). Using uniplex PCR and sequencing, 77.8% (21/27) of the discordant AdvanSure-positive and Real-Q RV II-negative results and 62.5% (10/16) of AdvanSure-negative and Real-Q RV II-positive results were confirmed as positive.

For ADV, IFN A, and PIV 3, the detection limits of the AdvanSure assay were 37.5, 5, and 1 copies/ μ L, respectively, and those

of the Real-Q RV II assay were 37.5, 50, and 500 copies/ μ L, respectively. In the analytical specificity test, all results were negative, and non-specific positive reactions were not observed in any assay.

Overall, the AdvanSure and Real-Q RV II assays demonstrated equivalent performance for detecting all viral targets. The vast majority of discordant results was associated with high threshold (Ct) values, suggesting low viral genome loads (data not shown). Among a total of eight discordant results for ETV, five were positive only with the AdvanSure assay; of these, none were confirmed to be positive by sequencing, while 80% (4/5) were HRV-positive, suggesting cross-reactivity between HRV and ETV in the AdvanSure assay. Previous studies have demonstrated that the high sequence similarity between HRV and ETV can cause cross-reactivity in molecular assays [4–7]. As we assayed a small number of ETV-positive samples, further studies are needed to confirm our results.

There were eight discrepant results for PIV 3. Notably, all discordant results that were positive only in the AdvanSure assay were confirmed to be positive by sequencing. The lower detection rate of PIV 3 by the Real-Q RV II assay than by the AdvanSure assay might be due to several factors, such as differences in target genes and primers and in the analytical sensitivities of the

Table 1. Detection of respiratory viruses using the AdvanSure RV plus real-time PCR and Real-Q RV II Detection assays

Virus	Percent agreement*				Kappa value	
	Positive		Negative		Observed kappa value	95% CI
	% (N)	95% CI	% (N)	95% CI		
ADV	100 (24/24)	82.8–100	100 (312/312)	98.5–100	1.00	
INF A	100 (24/24)	82.8–100	99.7 (311/312)	97.9–100	0.98	0.94–1.00
INF B	100 (12/12)	69.9–100	100 (324/324)	98.5–100	1.00	
PIV 1	100 (15/15)	74.7–100	100 (321/321)	98.5–100	1.00	
PIV 2	100 (15/15)	74.7–100	100 (321/321)	98.5–100	1.00	
PIV 3	100 (18/18)	78.1–100	97.5 (310/318)	94.9–98.8	0.81	0.68–0.94
HRV	84.4 (38/45)	69.9–93.0	99 (288/291)	96.8–99.7	0.87	0.79–0.95
RSV A	83.3 (15/18)	57.7–95.6	100 (318/318)	98.5–100	0.90	0.80–1.00
RSV B	100 (23/23)	82.2–100	100 (313/313)	98.5–100	1.00	
HMPV	92.9 (26/28)	75.0–98.8	99.7 (307/308)	97.9–100	0.94	0.87–1.00
CoV 229E/OC43	100 (28/28)	85.0–100	98.4 (303/308)	96.0–99.4	0.91	0.83–0.99
CoV NL63	100 (13/13)	71.7–100	99.4 (321/323)	97.5–99.9	0.93	0.82–1.00
BoV	93.8 (15/16)	67.7–99.7	99.4 (318/320)	97.5–99.9	0.90	0.80–1.00
ETV	50 (3/6)	13.9–86.1	98.5 (325/330)	96.3–99.4	0.42	0.09–0.75

*The Real-Q RV II assay was used as the comparative assay to calculate the positive percent agreement and negative percent agreement.

Abbreviations: ADV, adenovirus; INF A, influenza A virus; INF B, influenza B virus; PIV 1–3, parainfluenza virus types 1 to 3; HRV, human rhinovirus; RSV A, respiratory syncytial virus A; RSV B, respiratory syncytial virus B; HMPV, human metapneumovirus; CoV, coronavirus; BoV, bocavirus; ETV, enterovirus.

Table 2. Analysis of positive results obtained with the AdvanSure RV plus real-time PCR and Real-Q RV II Detection assays (N=312)

Virus	AdvanSure + / Real-Q RV II +	AdvanSure + /Real-Q RV II -		AdvanSure - /Real-Q RV II +	
		Samples (N)	Positive results in uniplex PCR and sequencing (N)	Samples (N)	Positive results in uniplex PCR and sequencing (N)
ADV	24	0	0	0	0
INF A	24	1	1	0	0
INF B	12	0	0	0	0
PIV 1	15	0	0	0	0
PIV 2	15	0	0	0	0
PIV 3	18	8	8	0	0
HRV	38	3	3	7	6
RSV A	15	0	0	3*	0
RSV B	23	0	0	0	0
HMPV	26	1	1	2	2
CoV 229E/OC43	28	5	4 [†]	0	0
CoV NL63	13	2	2	0	0
BoV	15	2	2	1	1
ETV	3	5*	0	3*	1

*One of these cases could not be confirmed by sequencing due to lack of residual samples; [†]These cases were confirmed as having CoV OC43 infection.

Abbreviations: AdvanSure, AdvanSure RV plus real-time PCR; Real-Q RV II, Real-Q RV II Detection assay; ADV, adenovirus; INF A, influenza A virus; INF B, influenza B virus; PIV 1-3, parainfluenza virus type 1 to 3; HRV, human rhinovirus; RSV A, respiratory syncytial virus A; RSV B, respiratory syncytial virus B; HMPV, human metapneumovirus; CoV, coronavirus; BoV, bocavirus; ETV, enterovirus.

assays [8, 9]. Thus, we conclude that the analytical sensitivity of the Real-Q RV II assay for PIV 3 is lower than that of the AdvanSure assay.

To the best of our knowledge, this is the first study comparing the performances of the AdvanSure and Real-Q RV II assays. Despite some discordant results, these assays yielded comparable results and were found to be potentially useful tools in clinical laboratories. Further studies with a large number of positive samples need to be performed in future.

ACKNOWLEDGEMENT

None.

AUTHOR CONTRIBUTION

Lee NY and Huh HJ initiated and designed the study and coordinated the drafting of the manuscript. Yun SA and Kim JY participated in sample collection and experiments. Chung YN and Yoo IY carried out data analysis and wrote the manuscript. Lee NY and Huh HJ supervised the study and reviewed the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

RESEARCH FUNDING

This study was supported by LG Chem, Korea. The sponsor was not involved in study design, data interpretation, or manuscript preparation.

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