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Performance Evaluation of the PowerChek SARS-CoV-2, Influenza A & B Multiplex Real-Time PCR Kit in Comparison with the BioFire Respiratory Panel

Tae Yeul Kim, M.D.[1], Ji-Youn Kim, M.T.[2], Hyang Jin Shim, M.T.[2], Sun Ae Yun, M.T.[2], Ja-Hyun Jang, M.D.[1], Hee Jae Huh, M.D.[1], Jong-Won Kim, M.D.[1], and Nam Yong Lee, M.D.[1]

[1]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Center for Clinical Medicine, Samsung Biomedical Research Institute, Samsung Medical Center, Seoul, Korea

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and influenza viruses may pose enormous challenges to our healthcare system. We evaluated the performance of the PowerChek SARS-CoV-2, Influenza A & B Multiplex Real-time PCR Kit (PowerChek; Kogene Biotech, Seoul, Korea) in comparison with the BioFire Respiratory Panels 2 and 2.1 (RP2 and RP2.1; bioMérieux, Marcy l'Étoile, France), using 147 nasopharyngeal swabs. The limit of detection (LOD) of the PowerChek assay was determined using SARS-CoV-2, influenza A, and B RNA standards. The LOD values of the PowerChek assay for SARS-CoV-2 and influenza A and B were 1.12, 1.24, and 0.61 copies/μL, respectively. The positive and negative percent agreements of the PowerChek assay compared with RP2 and RP2.1 were 97.5% (39/40) and 100% (107/107) for SARS-CoV-2; 100% (39/39) and 100% (108/108) for influenza A; and 100% (35/35) and 100% (112/112) for influenza B, respectively. The performance of the PowerChek assay was comparable to that of RP2 and RP2.1 for detecting SARS-CoV-2 and influenza A and B, suggesting its use in diagnosing SARS-CoV-2 and influenza infections.

Key Words: PowerChek SARS-CoV-2, Influenza A&B Multiplex Real-Time PCR Kit, SARS-CoV-2, Influenza, Real-time PCR, Performance, Agreement

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Corresponding author:
Hee Jae Huh, M.D., Ph.D.
Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel: +82-2-3410-1836
Fax: +82-2-3410-2719
E-mail: pmhhj77@gmail.com

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The emergence and rapid spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have resulted in an unprecedented public health crisis worldwide. As of November 7, 2021, SARS-CoV-2 has infected more than 249 million people worldwide, with more than five million deaths [1]. Rapid and accurate laboratory diagnosis is essential for controlling the SARS-CoV-2 pandemic [2]. Currently, molecular testing is the reference method for laboratory diagnosis of SARS-CoV-2 infection, and more than 200 molecular testing methods, most of which are based on real-time reverse transcription-polymerase chain reaction (rRT-PCR), have received emergency use authorization from the US Food and Drug Administration (FDA) [3-7].

During the SARS-CoV-2 pandemic, the circulation of influenza viruses (IFVs) may pose enormous challenges to physicians and public health officials as SARS-CoV-2 and IFV show similar clinical presentations, particularly in the early stage of infection [8]. This situation can be further complicated by the fact that SARS-CoV-2 and IFV co-infection has been reported, albeit infrequently [9-12]. Therefore, there is a pressing need for rapid and accurate diagnostic tests that can simultaneously detect SARS-CoV-2 and IFV.

To meet this need, the US Centers for Disease Control and Prevention and several commercial manufacturers have developed multiplex rRT-PCR assays for the simultaneous detection

of SARS-CoV-2, IFV A, and IFV B and have received emergency use authorization from the US FDA. The newly developed PowerChek SARS-CoV-2, Influenza A&B Multiplex Real-time PCR Kit (PowerChek; Kogene Biotech, Seoul, Korea) can detect and differentiate SARS-CoV-2, IFV A, and IFV B in nasopharyngeal swab (NPS) specimens and has recently obtained a Conformité Européenne mark. The PowerChek assay is a single-tube multiplex rRT-PCR assay capable of simultaneously detecting the open reading frame 1ab (*ORF1ab*) and envelope (*E*) genes of SARS-CoV-2, the matrix (*M*) gene of IFV A, and the nucleoprotein (*NP*) gene of IFV B. We evaluated the performance of the PowerChek assay in comparison with that of the BioFire Respiratory Panels 2 and 2.1 (RP2 and RP2.1, respectively; bioMérieux, Marcy l'Étoile, France). This study was approved by the Institutional Review Board of Samsung Medical Center, Seoul, Korea (approval number: 2020-12-061).

In this retrospective study, we analyzed 147 NPS specimens collected in viral transport media for routine IFV or SARS-CoV-2 testing at Samsung Medical Center between January 2017 and December 2020 (for IFV testing: between January 2017 and December 2018; for SARS-CoV-2 testing: between November and December 2020).

In our hospital, routine testing for SARS-CoV-2 is conducted using the PowerChek 2019-nCoV Real-time PCR Kit (Kogene Biotech), which tests for the *E* and RNA-dependent RNA polymerase (*RdRp*) genes, and IFV testing is conducted using the AdvanSure RV-Plus Real-Time RT-PCR assays for IFV A and B (LG Chem, Seoul, Korea). Specimens that tested positive during routine testing and spanned the range of positivity were selected for this study (Table 1). All specimens were stored at -70°C until analysis.

RNA was extracted from the NPS specimens using the QIAamp DSP Viral RNA Mini Kit (Qiagen, Hilden, Germany) or the Tianlong Libex automated nucleic acid extraction system (Tianlong Science and Technology, Xi'an, China) according to the manufacturers' instructions. Multiplex rRT-PCR was performed using the PowerChek assay per the manufacturer's instructions. Briefly, 5 μL of RNA was added to 15 μL of rRT-PCR master mix and 0.5 μL of internal control, resulting in a total volume of 20.5 μL. PCRs were run on the 7,500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) with the following cycling conditions: 50°C for 30 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A positive test result was defined as an exponential fluorescence curve that crossed the threshold line at or before 38 cycles (cycle threshold [Ct] ≤38). A specimen was considered positive for SARS-CoV-2 only when the test results for both SARS-CoV-2 target genes (*E* and *ORF1ab*) were positive. Specimens that were pos-

Table 1. Distribution of selected positive specimens according to Ct range

SARS-CoV-2			IFV		
Ct range*	*E* (N)	*RdRp* (N)	Ct range†	IFV A	IFV B
≤20.0	18	15	≤20.0	2	2
20.1-25.0	8	9	20.1-25.0	6	11
25.1-30.0	4	5	25.1-30.0	23	17
>30.0	10	11	>30.0	8	5
Total positive	40	40	Total positive	39	35

*Ct values were obtained by routine SARS-CoV-2 testing using the PowerChek 2019-nCoV Real-time PCR Kit (Kogene Biotech); †Ct values were calculated by adding 10 cycles to the raw Ct values obtained by routine IFV testing using the AdvanSure RV-plus real-time RT-PCR (LG Chem, Seoul, Korea), as this assay detects fluorescence signals from the 11th PCR cycle.
Abbreviations: *E*, envelope gene; *RdRp*, RNA-dependent RNA polymerase gene; Ct, cycle threshold; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; IFV, influenza virus.

Table 2. Comparison of the performance of the PowerChek and RP2 and RP2.1 assays

Comparator assay (target)		PowerChek assay		PPA (95% CI)	NPA (95% CI)	Kappa (95% CI)
		Positive	Negative			
RP2.1 (SARS-CoV-2)	Positive	39	1†	97.5% (86.8%-99.9%)	100% (96.6%-100%)	0.98 (0.95-1.00)
	Negative	0	107			
RP2/RP2.1 (IFV A)*	Positive	39	0	100% (91.0%-100%)	100% (96.6%-100%)	1.00 (1.00-1.00)
	Negative	0	108			
RP2/RP2.1 (IFV B)*	Positive	35	0	100% (90.0%-100%)	100% (96.8%-100%)	1.00 (1.00-1.00)
	Negative	0	112			

*In the RP2 and RP2.1 assays, the test results for IFV A and B were all identical; †In the PowerChek assay, one specimen repeatedly yielded an inconclusive result (positive for *ORF1ab*, but negative for *E*). To calculate the agreement, this specimen was considered negative.
Abbreviations: PPA, positive percent agreement; NPA, negative percent agreement; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; IFV, influenza virus.

Table 3. Assessment of the LOD of the PowerChek assay

Concentration	Replicates	PowerChek assay			
		SARS-CoV-2		IFV A *M* gene	IFV B *NP* gene
		E gene	*ORF1ab* gene		
10 copies/μL	3	31.80	32.58	30.56	31.30
		31.32	32.84	30.65	31.30
		31.62	32.63	31.02	31.03
5 copies/μL	8	32.33	34.28	Not done	Not done
		33.09	34.49	Not done	Not done
		33.19	34.19	Not done	Not done
		33.77	36.59	Not done	Not done
		33.27	34.54	Not done	Not done
		32.91	34.54	Not done	Not done
		33.26	34.09	Not done	Not done
		33.63	34.76	Not done	Not done
1 copy/μL	11	34.01	36.20	33.40	33.97
		34.53	36.04	33.78	34.32
		33.65	36.09	33.83	34.67
		35.91	36.30	34.12	34.69
		36.94	35.57	34.97	34.77
		34.83	37.86	35.14	34.95
		Not detected	35.27	35.54	35.01
		Not detected	35.72	35.75	35.06
		35.89	Not detected	35.93	35.15
		Not detected	Not detected	Not detected	35.42
		Not detected	Not detected	Not detected	35.99
0.5 copies/μL	8	35.94	Not detected	34.93	35.02
		36.55	Not detected	35.60	35.67
		Not detected	36.45	36.18	36.49
		Not detected	36.95	36.72	36.71
		Not detected	37.07	36.86	36.93
		Not detected	Not detected	37.08	37.04
		Not detected	Not detected	Not detected	38.38
		Not detected	Not detected	Not detected	Not detected
0.1 copies/μL	8	Not done	Not done	35.97	36.69
		Not done	Not done	Not detected	36.77
		Not done	Not done	Not detected	Not detected
		Not done	Not done	Not detected	Not detected
		Not done	Not done	Not detected	Not detected
		Not done	Not done	Not detected	Not detected
		Not done	Not done	Not detected	Not detected
		Not done	Not done	Not detected	Not detected

Abbreviations: LOD, limit of detection; NP, nucleoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; IFV, influenza virus.

itive for only one target gene were considered inconclusive and were retested; if the result remained inconclusive, it was reported as inconclusive. Specimens showing a positive test result for the *M* gene were considered positive for influenza A, and specimens exhibiting a positive test result for the *NP* gene were considered positive for influenza B.

RP2 is an FDA-cleared, sample-to-answer multiplex PCR assay that detects 22 respiratory pathogens, including IFV A (subtypes H1, H1-2009, and H3) and IFV B, in NPS specimens. RP2.1 (also FDA-cleared) additionally detects the SARS-CoV-2 spike protein (*S*) and membrane protein (*M*) genes. NPS specimens were tested using the RP2 and RP2.1 assays per the manufacturer's instructions. Specimens with discordant results for SARS-CoV-2 between the PowerChek and RP2.1 assays were confirmed using the Xpert Xpress SARS-CoV-2 assay kit (Cepheid, Sunnyvale, CA, USA).

SARS-CoV-2 and IFV A and B *in vitro* transcripts of known copy number (AcroMetrix Coronavirus 2019 RNA Control; Thermo Fisher Scientific, and AmpliRun IFV A H1 and IFV B RNA Control; Vircell, Granada, Spain) were used in limit of detection (LOD) assessment. The RNA standards were serially diluted, and each dilution was tested in multiple replicates using the PowerChek assay. Probit regression analysis was used to estimate the LOD.

Positive percent agreement and negative percent agreement between the PowerChek assay and RP2.1 for SARS-CoV-2 were 97.5% (39/40) and 100% (107/107), respectively (Table 2). For IFV, the PowerChek assay yielded results identical to the RP2 and RP2.1 assays. Kappa values ranged from 0.98 (SARS-CoV-2) to 1.00 (IFV A and B), indicating nearly perfect agreement. Only one specimen gave discordant results between the PowerChek and comparator assays. This specimen tested positive for SARS-CoV-2 by the RP2.1 assay, whereas the PowerChek assay result was repeatedly inconclusive (positive for *ORF1ab* [Ct ≥36.0], but negative for *E*). This specimen was tested additionally with the Xpert Xpress SARS-CoV-2 assay, which gave a presumptive positive result, with only *E* detected (Ct=32.8). The LOD assessment results are shown in Table 3. The LOD values of the PowerChek assay for SARS-CoV-2 and IFV A and B were 1.12, 1.24, and 0.61 copies/μL, respectively, which were higher than the manufacturer-claimed LOD values of RP2/RP2.1 assays (SARS-CoV-2: 0.5 copies/μL for heat-inactivated virus and 0.16 copies/μL for infectious virus; IFV A H1: 0.14 copies/μL; IFV B: 0.034 copies/μL).

Multiplex assays for the simultaneous detection of SARS-CoV-2 and other respiratory viruses, including IFV, are currently commercially available [13-19]. Most of these assays are run on automated sample-to-answer platforms, allowing near-patient testing. RP2.1 and the Xpert Xpress SARS-CoV-2/Flu/RSV assay are two such assays, and their performance has been evaluated [14, 15, 17, 18]. These assays can be performed in a random-access mode, providing timely test results to physicians; however, they may not be suitable for high-volume laboratories due to the limited testing capacity. In contrast, the PowerChek assay has a high-throughput capacity (up to 96 specimens per batch) and is, therefore, well-suited to high-volume laboratories. Particularly when SARS-CoV-2 and IFV are prevalent, high-throughput multiplex assays such as the PowerChek assay will be urgently needed.

The only one discordant specimen obtained had high Ct values for *ORF1ab*, suggesting that the SARS-CoV-2 viral load was extremely low. Indeed, analysis of this specimen with a third assay, Xpert Xpress SARS-CoV-2, gave a presumptive positive result as it was *E*-positive, but *N2*-negative. Overall, the performance of PowerChek assay in detecting SARS-CoV-2 and IFV A and B was equivalent to that of RP2 and RP2.1. The PowerChek assay can be used in most laboratories with various real-time PCR platforms, and thus, provides a high-throughput and robust option for clinical laboratories.

A major limitation of this single-center study was its retrospective design. A prospective study was not possible as, in our hospital, IFV-positive specimens have not been found during the SARS-CoV-2 pandemic. Thus, stored specimens were selectively included to obtain a sufficient number of positive specimens.

In conclusion, the performance of the PowerChek assay is comparable with that of the RP2 and RP2.1 assays. Our results indicate that the PowerChek assay is a useful diagnostic tool for the detection of SARS-CoV-2 and IFV.

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AUTHOR CONTRIBUTIONS

Huh HJ designed the study, supervised the data analyses, and reviewed the manuscript. Kim TY analyzed the data and wrote the manuscript. Kim J, Shim HJ, and Yun SA participated in experiments. Jang J, Kim J, and Lee NY reviewed the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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ORCID

Tae Yeul Kim https://orcid.org/0000-0002-6405-5305
Ji-Youn Kim https://orcid.org/0000-0002-9206-7338
Hyang Jin Shim https://orcid.org/0000-0002-9387-4932
Sun Ae Yun https://orcid.org/0000-0002-8104-3496
Ja-Hyun Jang https://orcid.org/0000-0003-0516-4947
Hee Jae Huh https://orcid.org/0000-0001-8999-7561
Jong-Won Kim https://orcid.org/0000-0002-0708-9242
Nam Yong Lee https://orcid.org/0000-0003-3688-0145

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