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Comparison of the PowerChek SARS-CoV-2, Influenza A&B, RSV Multiplex Real-time PCR Kit and BioFire Respiratory Panel 2.1 for simultaneous detection of SARS-CoV-2, influenza A and B, and respiratory syncytial virus

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ABSTRACT

The potential co-circulation of SARS-CoV-2, influenza, and respiratory syncytial virus (RSV) could pose an unprecedented challenge to healthcare systems worldwide. Here, we compared the performance of the PowerChek SARS-CoV-2, Influenza A&B, RSV Multiplex Real-time PCR Kit (PowerChek) for simultaneous detection of SARS-CoV-2, influenza A and B, and respiratory syncytial virus with that of BioFire Respiratory Panel 2.1 (RP2.1) using 175 nasopharyngeal swab (NPS) specimens. Positive percent agreement and negative percent agreement of the PowerChek assay compared to RP2.1 were as follows: 100 % (40/40) and 100 % (135/135) for SARS-CoV-2; 100 % (39/39) and 100 % (136/136) for influenza A; 100 % (35/35) and 100 % (140/140) for influenza B; and 93.1 % (27/29) and 100 % (146/146) for RSV, respectively. The limit of detection (LOD) was accessed using RNA standards for each virus, and the LOD values of the PowerChek assay for SARS-CoV-2, influenza A and B, and RSV were 0.36, 1.24, 0.09, and 0.63 copies/μL, respectively. Our results demonstrate that the PowerChek assay is sensitive and accurate for detection of SARS-CoV-2, influenza A and B, and RSV, suggesting that this assay can be a valuable diagnostic tool when SARS-CoV-2, influenza, and RSV are co-circulating.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly, resulting in characterization of this outbreak as a pandemic. During this era of the SARS-CoV-2 pandemic, co-circulation of influenza and respiratory syncytial virus (RSV) could pose a challenge to healthcare providers as these viral infections have overlapping clinical presentations (Li et al., 2020; Solomon et al., 2020; Zayet et al., 2020). Further complicating the situation is that co-infection of SARS-CoV-2 and other respiratory viruses, including influenza and RSV, is possible (Burrel et al., 2021; Cuadrado-Payan et al., 2020; Ding et al., 2020; Kim et al., 2020; Ma et al., 2020; Wu et al., 2020; Zhang et al., 2020); therefore, clinical laboratories need rapid and accurate diagnostic assays that can detect and differentiate among SARS-CoV-2, influenza, and RSV.

The PowerChek SARS-CoV-2, Influenza A&B, RSV Multiplex Real-time PCR Kit (PowerChek; Kogene Biotech, Seoul, Korea) is a newly developed molecular diagnostic assay that can detect and differentiate SARS-CoV-2, influenza A and B, and RSV in nasopharyngeal swab (NPS) specimens, which has recently received CE-IVD marking. The PowerChek assay is a real-time reverse transcription-polymerase chain reaction (rRT-PCR) test capable of simultaneously detecting the open reading frame 1ab (ORF1ab) and envelope (E) genes of SARS-CoV-2, the matrix (M) gene of influenza A, the nucleoprotein (NP) gene of influenza B, and the nucleocapsid (N) gene of RSV. In this study, we evaluated the performance of the PowerChek assay and compared it to that of BioFire Respiratory Panel 2.1 (RP2.1; bioMérieux, Marcy l'Etoile, France).

A total of 175 NPS specimens in viral transport media that had been collected for routine influenza, RSV, or SARS-CoV-2 testing between November 2016 and December 2020 at Samsung Medical Center were used for this retrospective study. These specimens included 40 specimens that tested positive for SARS-CoV-2 by the PowerChek 2019-nCoV Real-time PCR Kit (Kogene Biotech) and 39, 35, and 28 specimens that tested positive for influenza A, influenza B, and RSV, respectively, by the AdvanSure RV-plus real-time RT-PCR (LG Chem, Seoul, Korea) (Supplementary Table 1). All specimens were stored at −70 °C until tested

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using the PowerChek and RP2.1 assays.

RNA was extracted from 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 Co., Ltd., Xi'an, China) according to the manufacturers' instructions. The PowerChek assay comprises two reaction tubes and was performed according to the manufacturer's instructions. Briefly, 5 μL of extracted RNA was added to 15 μL of rRT-PCR master mix, resulting in a total volume of 20 μL. The rRT-PCR was performed using a 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) with the following cycling conditions: 1 cycle at 50 °C for 30 min and 1 cycle at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. 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).

The RP2.1 assay was used as a comparator assay and was performed according to the manufacturer's instructions. Specimens with discordant results between the PowerChek and RP2.1 were further tested using the Allplex Respiratory Panel 1 (Allplex; Seegene, Seoul, Republic of Korea) for discrepancy resolution.

SARS-CoV-2, influenza A and B, and RSV *in vitro* transcripts of known concentration (AcroMetrix Coronavirus 2019 RNA Control [Thermo Fisher Scientific, Fremont, CA]; AmpliRun Influenza A H1, Influenza B, and RSV subtype A RNA Control [Vircell, Granada, Spain]) were used for analytical sensitivity evaluation. These RNA standards were serially diluted, and multiple replicates of each dilution were tested using the PowerChek assay. The limit of detection (LOD) was calculated using Probit regression analysis. Analytical specificity was evaluated using 20 respiratory virus strains (Table 1).

For SARS-CoV-2, the PPA and NPA between the PowerChek assay and RP2.1 were 100 % (40/40) and 100 % (135/135), respectively (Table 2). For influenza A and B, the PPA and NPA between the PowerChek assay and RP2.1 were as follows: 100 % (39/39) and 100 % (136/136) for influenza A and 100 % (35/35) and 100 % (140/140) for influenza B. For RSV, the PPA and NPA between the PowerChek assay and RP2.1 for RSV were 93.1 % (27/29) and 100 % (146/146), respectively. Cohen's Kappa values ranged from 0.96 (RSV) to 1.00 (SARS-CoV-2 and influenza A and B), which suggests almost perfect agreement. Two specimens produced discordant results between the PowerChek assay and RP2.1 for RSV (Table 3). They were PowerChek-negative and RP2.1-positive for RSV. After discrepancy resolution, one specimen was confirmed as positive for RSV, and this sample's high Ct value (39.5) indicates a low RSV viral load in the specimen. The LOD values of the PowerChek assay for SARS-CoV-2, influenza A and B, and RSV were 0.36, 1.24, 0.09, and 0.63 copies/μL, respectively (Table 4), which were comparable to or higher than the claimed LOD values of the RP2.1 assay in the package insert (SARS-CoV-2: 0.5 copies/μL for heat-inactivated virus and 0.16 copies/μL for infectious virus; influenza A H1: 0.14 copies/μL; influenza B: 0.034 copies/μL; RSV: 0.009 copies/μL). In the analytical specificity study, the PowerChek assay detected only its intended targets (SARS-CoV-2, influenza A and B, and RSV) and showed no cross-reactivity with other respiratory viruses (Table 1).

Table 1
Analytic specificity of the PowerChek assay.

Virus	Source (code number)	Result
SARS-CoV-2	NCCP (43326)	SARS-CoV-2 positive
SARS-CoV	EVAg (011N-03868)	Negative
Human coronavirus 229E	KBPV (VR-9)	Negative
Human coronavirus OC43	KBPV (VR-8)	Negative
Human coronavirus NL63	KBPV (VR-88D)	Negative
Human coronavirus HKU1	ATCC (VR-3262SD)	Negative
Influenza A H1N1	Vircell (MBC082)	Influenza A positive
Influenza A H3	Vircell (MBC029)	Influenza A positive
Influenza B	Vircell (MBC030)	Influenza B positive
RSV type A	Vircell (MBC041)	RSV positive
RSV type B	Vircell (MBC083)	RSV positive
Adenovirus	Vircell (MBC001)	Negative
Metapneumovirus	KBPV (VR-87)	Negative
Parainfluenza virus type 1	Vircell (MBC037)	Negative
Parainfluenza virus type 2	Vircell (MBC038)	Negative
Parainfluenza virus type 3	Vircell (MBC039)	Negative
Parainfluenza virus type 4	KBPV (VR-70)	Negative
Enterovirus 68	Vircell (MBC125)	Negative
Enterovirus 71	Vircell (MBC019)	Negative
Rhinovirus	Vircell (MBC091)	Negative

NCCP, National Culture Collection for Pathogens; EVAg, European Virus Archive; KBPV, Korea Bank for Pathogenic Viruses; ATCC, American Type Culture Collection.

Currently, several multiplex rRT-PCR assays for simultaneous detection of respiratory viruses including SARS-CoV-2 are commercially available (Chung et al., 2021; Creager et al., 2020; Eckbo et al., 2021; Jarrett et al., 2021; Leung et al., 2021; Mostafa et al., 2020; Visseaux et al., 2020). Most of these assays have been developed by adding SARS-CoV-2 testing to existing multiplex assays for detection of other respiratory viruses including influenza and RSV. The RP2.1, the Xpert Xpress SARS-CoV-2/Flu/RSV (Cepheid, Sunnyvale, CA, USA), the QIAstat-Dx respiratory SARS-CoV-2 panel (Qiagen, Hilden, Germany), and the ePlex Respiratory Pathogen Panel 2 (GenMark Diagnostics, Carlsbad, CA, USA) are such assays, and their performance has been assessed in previous studies (Creager et al., 2020; Eckbo et al., 2021; Jarrett et al., 2021; Leung et al., 2021; Mostafa et al., 2020; Visseaux et al., 2020). Although these random-access assays make test results available to clinicians in a timely manner, they have a relatively low, albeit scalable, throughput and might not be suitable for high-volume laboratories. On the other hand, the PowerChek assay is a high-throughput batch testing, suitable for laboratories performing a large number of assays.

Limitations of this single-center study are its retrospective design and small sample size. A prospective study was not feasible as influenza- and RSV-positive samples have rarely been found in our hospital during the SARS-CoV-2 pandemic. Therefore, stored clinical specimens were selectively included to evaluate the performance of the PowerChek assay.

According to our study, the performance of the PowerChek assay was comparable to that of the RP2.1 assay in detecting SARS-CoV-2, influenza A and B, and RSV. Our results indicate that the PowerChek assay is a useful diagnostic tool for simultaneous detection of SARS-CoV-2, influenza, and RSV.

Ethical statement

The study protocol was reviewed and approved by the Institutional Review Board of Samsung Medical Center (IRB no. SMC 2020-12-061).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Tae Yeul Kim: Formal analysis, Writing - original draft. **Ji-Youn Kim:** Investigation. **Hyang Jin Shim:** Investigation. **Sun Ae Yun:**

Table 2
Agreement between the PowerChek assay and RP2.1 assay.

Target	RP2.1	PowerChek assay		PPA (95 % CI)	NPA (95 % CI)	Kappa value (95 % CI)
		Positive	Negative			
SARS-CoV-2	Positive	40	0	100 %	100 %	1.00
	Negative	0	135	(91.2–100 %)	(97.3–100 %)	
Influenza A	Positive	39	0	100 %	100 %	1.00
	Negative	0	136	(91.0–100 %)	(97.3–100 %)	
Influenza B	Positive	35	0	100 %	100 %	1.00
	Negative	0	140	(90.0–100 %)	(97.4–100 %)	
RSV	Positive	27	2	93.1 %	100 %	0.96
	Negative	0	146	(77.2–99.2 %)	(97.5–100 %)	(0.90–1.00)

Table 3
Details of the two specimens with discordant results.

No.	PowerChek assay result (Ct value)			RP2.1 result			Discrepancy resolution (Allplex assay)	
	SARS-CoV-2	Influenza	RSV	SARS-CoV-2	Influenza	RSV	RSV	Ct value*
1	Negative	Influenza A (29.4)	Negative	Negative	Influenza A	Positive	Negative	–
2	Negative	Influenza B (30.8)	Negative	Negative	Influenza B	Positive	Positive	39.5

* A positive result was defined as Ct value $\leq$ 42.

Table 4
Assessment of limit of detection of the PowerChek assay.

Target	SARS-CoV-2			Influenza A			Influenza B			RSV		
Concentration	copies/μL	Replicates	Detected (mean Ct)*	copies/μL	Replicates	Detected (mean Ct)	copies/μL	Replicates	Detected (mean Ct)	copies/μL	Replicates	Detected (mean Ct)
#1	1	20	20 (36.8/34.0)	1	20	17 (36.0)	0.5	20	20 (35.8)	1	20	20 (34.2)
#2	0.5	20	20 (37.2/34.8)	0.5	20	13 (36.6)	0.1	20	20 (35.6)	0.5	20	17 (34.9)
#3	0.25	20	16 (37.6/36.1)	0.25	20	7 (37.1)	0.05	20	4 (37.3)	0.2	20	10 (36.5)
#4	0.125	20	12 (37.7/36.2)	0.125	20	5 (37.0)	0.025	20	1 (36.8)	0.1	20	7 (36.3)
#5										0.05	20	6 (36.9)
#6										0.025	20	3 (37.1)
Probit LOD (copies/μL)	0.36			1.24			0.09			0.63		

* Numbers before and after the slash indicate the Ct values of the E and ORF1ab genes, respectively.

Investigation. **Ja-Hyun Jang:** Writing - review & editing. **Hee Jae Huh:** Conceptualization, Formal analysis, Supervision, Writing - review & editing. **Jong-Won Kim:** Writing - review & editing. **Nam Yong Lee:** Writing - review & editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2021.114304.

References

Burrel, S., Hausfater, P., Dres, M., Pourcher, V., Luyt, C.E., Teyssou, E., Soulie, C., Calvez, V., Marcelin, A.G., Boutolleau, D., 2021. Co-infection of SARS-CoV-2 with other respiratory viruses and performance of lower respiratory tract samples for the diagnosis of COVID-19. Int. J. Infect. Dis. 102, 10–13.

Chung, H.Y., Jian, M.J., Chang, C.K., Lin, J.C., Yeh, K.M., Chen, C.W., Chiu, S.K., Wang, Y.H., Liao, S.J., Li, S.Y., et al., 2021. Novel dual multiplex real-time RT-PCR assays for the rapid detection of SARS-CoV-2, influenza A/B, and respiratory syncytial virus using the BD MAX open system. Emerg. Microbes Infect. 10, 161–166.

Creager, H.M., Cabrera, B., Schnaubelt, A., Cox, J.L., Cushman-Vokoun, A.M., Shakir, S. M., Tardif, K.D., Huang, M.L., Jerome, K.R., Greninger, A.L., et al., 2020. Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2. J. Clin. Virol. 129, 104538.

Cuadrado-Payan, E., Montagud-Marrahi, E., Torres-Elorza, M., Bodro, M., Blasco, M., Poch, E., Soriano, A., Pineiro, G.J., 2020. SARS-CoV-2 and influenza virus co-infection. Lancet 395, e84.

Ding, Q., Lu, P., Fan, Y., Xia, Y., Liu, M., 2020. The clinical characteristics of pneumonia patients coinfected with 2019 novel coronavirus and influenza virus in Wuhan, China. J. Med. Virol. 92, 1549–1555.

Eckbo, E.J., Locher, K., Caza, M., Li, L., Lavergne, V., Charles, M., 2021. Evaluation of the BioFire® COVID-19 test and Respiratory Panel 2.1 for rapid identification of SARS-CoV-2 in nasopharyngeal swab samples. Diagn. Microbiol. Infect. Dis. 99, 115260.

Jarrett, J., Uhteg, K., Forman, M.S., Hanlon, A., Vargas, C., Carroll, K.C., Valsamakis, A., Mostafa, H.H., 2021. Clinical performance of the GenMark Dx ePlex respiratory pathogen panels for upper and lower respiratory tract infections. J. Clin. Virol. 135, 104737.

Kim, D., Quinn, J., Pinsky, B., Shah, N.H., Brown, I., 2020. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. JAMA 323, 2085–2086.

Leung, E.C., Chow, V.C., Lee, M.K., Tang, K.P., Li, D.K., Lai, R.W., 2021. Evaluation of the Xpert Xpress SARS-CoV-2/Flu/RSV assay for simultaneous detection of SARS-CoV-2, influenza A/B and respiratory syncytial viruses in nasopharyngeal specimens. J. Clin. Microbiol.

Li, Y., Wang, J., Wang, C., Yang, Q., Xu, Y., Xu, J., Li, Y., Yu, X., Zhu, H., Liu, J., 2020. Characteristics of respiratory virus infection during the outbreak of 2019 novel coronavirus in Beijing. Int. J. Infect. Dis. 96, 266–269.

Ma, L., Wang, W., Le Grange, J.M., Wang, X., Du, S., Li, C., Wei, J., Zhang, J.N., 2020. Coinfection of SARS-CoV-2 and other respiratory pathogens. Infect. Drug Resist. 13, 3045–3053.

Mostafa, H.H., Carroll, K.C., Hicken, R., Berry, G.J., Manji, R., Smith, E., Rakeman, J.L., Fowler, R.C., Leelawong, M., Butler-Wu, S.M., et al., 2020. Multi-center evaluation of the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test. J. Clin. Microbiol.

Solomon, D.A., Sherman, A.C., Kanjilal, S., 2020. Influenza in the COVID-19 era. JAMA 324, 1342–1343.

Visseaux, B., Le Hingrat, Q., Collin, G., Bouzid, D., Lebourgeois, S., Le Pluart, D., Deconinck, L., Lescure, F.X., Lucet, J.C., Bouadma, L., et al., 2020. Evaluation of the QIAstat-Dx respiratory SARS-CoV-2 panel, the first rapid multiplex PCR commercial assay for SARS-CoV-2 detection. J. Clin. Microbiol. 58.

Wu, X., Cai, Y., Huang, X., Yu, X., Zhao, L., Wang, F., Li, Q., Gu, S., Xu, T., Li, Y., et al., 2020. Co-infection with SARS-CoV-2 and influenza A virus in patient with pneumonia, China. Emerg. Infect. Dis. 26, 1324–1326.

Zayet, S., Kadiane-Oussou, N.J., Lepiller, Q., Zahra, H., Royer, P.Y., Toko, L., Gendrin, V., Klopfenstein, T., 2020. Clinical features of COVID-19 and influenza: a comparative study on Nord Franche-Comte cluster. Microbes Infect. 22, 481–488.

Zhang, D.D., Acree, M.E., Ridgway, J.P., Shah, N., Hazra, A., Ravichandran, U., Kumar, M., 2020. Characterizing coinfection in children with COVID-19: a dual center retrospective analysis. Infect. Control Hosp. Epidemiol. 1–3.