Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
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

Tae Yeul Kim a, Ji-Youn Kim b, Hyang Jin Shim b, Sun Ae Yun b, Ja-Hyun Jang a, Hee Jae Huh a,*, Jong-Won Kim a, Nam Yong Lee a

a Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
b Center for Clinical Medicine, Samsung Biomedical Research Institute, Samsung Medical Center, Seoul, Republic of Korea

ARTICLE INFO

Keywords:
PowerChek
SARS-CoV-2
COVID-19
Influenza
RSV
Real-time PCR

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 assessed 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.
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 RT-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 [Virecall, 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 Pow- erChek 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).

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.

**Funding**

This research was supported by the Kogene Biotech and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HW20C2130). The sponsor had no involvement in the study design, data interpretation, or preparation of the manuscript.

**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          | PowerChek assay | RP2.1  |
|-----------------|-----------------|--------|
|                 | Positive        | Negative|
| SARS-CoV-2      | 100 %           | 100 %  |
| Influenza A     | 0 %             | 91.2–100 %|
| Influenza B     | 0 %             | 91.0–100 %|
| RSV             | 93.1 %          | 100 %  |

Table 3
Details of the two specimens with discordant results.

| No. | PowerChek assay result (Ct value) | RP2.1 result | Discrepancy resolution (Allplex assay) |
|-----|----------------------------------|--------------|---------------------------------------|
| 1   | Negative                         | Influenza A (29.4) | Positive                              |
| 2   | Negative                         | Influenza B (30.8) | Positive                              |

* A positive result was defined as Ct value ≤ 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) | Replicates Detected (mean Ct) | Replicates Detected (mean Ct) |
| #1              | 1          | 20 (36.8/34.0) | 17 (36.0) | 0.5 | 20 | 20 (35.8) |
| #2              | 0.5        | 20 (37.2/34.8) | 13 (36.6) | 0.1 | 20 | 20 (35.6) |
| #3              | 0.25       | 20 (37.6/36.1) | 7 (37.1) | 0.05 | 20 | 4 (37.3) |
| #4              | 0.125      | 20 (37.7/36.2) | 5 (37.0) | 0.025 | 20 | 1 (36.8) |
| #5              |            |              |            | 0.1 | 20 | 7 (36.3) |
| #6              |            |              |            | 0.5 | 20 | 6 (36.9) |
| Probit LOD      | 0.36       | 1.24         | 0.09        | 0.025 | 20 | 3 (37.1) |

* 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 dohttps://doi.org/10.1016/j.jviromet.2021.114304.

References

Burrell, S., Haasnoot, P., Dren, M., Pourcher, V., Layt, C.E., Teysou, E., Soulie, C., Calvès, V., Marcellin, A.G., Beutelspaen, 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., Creager, H.M., Cabrera, B., Schnaubelt, A., Cox, J.L., Cushman-Vokoun, A.M., Shakir, S.M., 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.

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.

Eckroth, F.J., Loecher, 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., Usteg, K., Forman, M.S., Hanlon, A., Vargas, C., Carroll, K.C., Valmaggis, 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, L., 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., Xu, Z., 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, 3045–3052.

Montafa, H.H., Carroll, K.C., Hicken, R., Berry, G.J., Manji, R., Smith, E., Rakeman, J.L., Fowler, R.C., Leelaawong, 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.

Viseux, B., Le Hingrat, Q., Collin, G., Bouzid, D., Lebourgé, S., Le Pluant, D., Deconinck, L., Lescure, F.X., Lucet, J.C., Boudama, L., et al., 2020. Evaluation of the QHastat-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.