AccuPower® RV1 Real-Time RT-PCR 키트와 AccuPower® RV1 Multiplex 키트의 SARS-CoV-2 및 인플루엔자 바이러스 검출에 대한 평가

Evaluation of the AccuPower® RV1 Real-Time RT-PCR Kit and the AccuPower® RV1 Multiplex Kit for SARS-CoV-2 and Influenza Virus Detection

김만진^{1,2} · 박현웅³ · 전유라⁴ · 신호섭¹ · 조성임¹ · 김보람¹ · 이지수¹ · 박성섭¹ · 성문우¹

Man Jin Kim, M.D.^{1,2}, Hyunwoong Park, M.D.³, You La Jeon, M.D.⁴, Ho Seob Shin, M.T.¹, Sung Im Cho, M.T.¹, Boram Kim, M.D.¹, Jee-Soo Lee, M.D.¹, Sung Sup Park, M.D.¹, Moon-Woo Seong, M.D.¹

서울대학교병원 진단검사의학과¹, 서울대학교병원 임상유전체의학과², 서울대학교병원운영 서울특별시보라매병원 진단검사의학과³, 녹십자의료재단⁴

Departments of Laboratory Medicine¹ and Genomic Medicine², Seoul National University Hospital, Seoul; Department of Laboratory Medicine³, Seoul Metropolitan Government-Seoul National University Boramae Medical Center, Seoul; Department of Laboratory Medicine⁴, Green Cross Laboratories, Yongin, Korea

Background: The AccuPower® RV1 Real-Time RT-PCR Kit (Bioneer, Korea) and AccuPower® RV1 Multiplex Kit (Bioneer) are one-step real-time reverse transcription PCR assays for detecting severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and influenza A and B.

Methods: We clinically evaluated the AccuPower® RV1 Real-Time RT-PCR Kit and AccuPower® RV1 Multiplex Kit by comparing their results for 1,098 clinical samples. The presence of SARS-CoV-2 was confirmed using the Allplex™ 2019-nCoV Assay (Seegene, Korea) and Standard M nCoV Real-Time Detection Kit (SD Biosensor, Korea). Influenza viruses were detected using the Allplex™ Respiratory Panel 1 (Seegene).

Results: The comparative positive and negative agreement values of the AccuPower® RV1 Real-Time RT-PCR Kit for SARS-CoV-2 and influenza A and B were 100%. The positive agreement of the AccuPower® RV1 Multiplex Kit was 100% for SARS-CoV-2 and 98.77% for influenza A and B. The kappa values for SARS-CoV-2 and influenza A and B were > 0.99. SARS-CoV-2 was evaluated using both sputum and nasopharyngeal or oropharyngeal swabs. There was no difference in the detection rates for each type.

Conclusions: The findings confirm the clinically comparable performances of the AccuPower® RV1 Real-Time RT-PCR Kit and the AccuPower® RV1 Multiplex Kit.

Key Words: SARS-CoV-2, Influenza virus, Multiplex real-time PCR, Clinical evaluation

INTRODUCTION

Corresponding author: Moon-Woo Seong, M.D., Ph.D.

<https://orcid.org/0000-0003-2954-3677>

Department of Laboratory Medicine, Seoul National University Hospital, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea

Tel: +82-2-2072-4180, Fax: +82-2-747-0359, E-mail: mwseong@snu.ac.kr

Received: June 5, 2021

Revision received: July 31, 2021

Accepted: August 9, 2021

This article is available from <https://www.labmedonline.org>

© 2021, Laboratory Medicine Online

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Coronavirus disease 2019 (COVID-19) was first detected in Wuhan, Hubei Province, China. The disease is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [1, 2].

To date, there have been over 100 million cases in the ongoing SARS-CoV-2 pandemic, with over 2 million deaths globally, according to the COVID-19 dashboard, Center for Systems Science and Engineering, Johns Hopkins University. We cannot accurately diagnose patients with COVID-19 based on the observable symptoms alone because these are nonspecific, such as fever, cough, sputum production, and shortness of breath, and are shared by other respiratory infections [3]. Influenza virus is a common cause

of respiratory infections. Influenza and SARS-CoV-2 infections present similar symptoms and modes of transmission, including transmission through contact, droplets, and fomites [4].

Alveolar type II cells (AT2 pneumocytes), the primary site of influenza replication, especially influenza A, have been implicated as targets for SARS-CoV-2 infection [5]. There is scant evidence regarding the interaction between COVID-19 and influenza. One case report described that symptoms worsened when patients were co-infected with SARS-CoV-2 and influenza A [6]. Another report described rapid respiratory deterioration in four patients infected with SARS-CoV-2 and influenza A or B [7]. Moreover, simultaneous infection with SARS-CoV-2 and other respiratory pathogens may influence the morbidity and prognosis in patients [8]. It is important to accurately detect SARS-CoV-2 and influenza viruses, including possible coinfections in the community [9].

Reverse-transcription PCR (RT-PCR) is highly sensitive and specific and has become the standard test for detecting respiratory viruses, including SARS-CoV-2 [8]. This study aimed to evaluate a multiplex PCR to detect SARS-CoV-2 and influenza A and B viruses using the ExiStation™ system (Bioneer, Daejeon, Korea).

MATERIALS AND METHODS

1. Clinical specimens

This study was approved by the Institutional Review Board of the Seoul National University Hospital. The study included 1,098 clinical respiratory specimens obtained from the Seoul National University Hospital, Seoul National University Boramae Medical Center, and Green Cross Laboratory. A total of 378 sputum samples and 720 nasopharyngeal/oropharyngeal swabs were collected from patients infected with SARS-CoV-2 or influenza. All the sputum samples and 382 nasopharyngeal or oropharyngeal swabs were analyzed for the presence of SARS-CoV-2. The remaining 338 nasopharyngeal or oropharyngeal swabs were tested for influenza A and B.

Clinical testing was performed using retrospective clinical samples. We analyzed the samples using the Allplex™ 2019-nCoV Assay (Seegene, Seoul, Korea) and STANDARD M nCoV Real-Time Detection kit (SD Biosensor, Suwon, Korea) for SARS-CoV-2, and Allplex™ Respiratory Panel 1 (Seegene) for influenza viruses. All specimens were stored at -70°C until testing with the AccuPower® RV1 Real-Time RT-PCR Kit (Bioneer) and the AccuPower® RV1

Multiplex Kit (Bioneer). The samples were not frozen or thawed more than once.

2. AccuPower® RV1 Real-Time RT-PCR Kit and AccuPower® RV1 Multiplex Kit

The AccuPower® RV1 Kits can detect SARS-CoV-2, influenza A (Influenza A-H1, Influenza A-H3N2, and Influenza A-pdm09), and influenza B with different Ct values. The test was performed using an Exicycler™ 96 (Bioneer) for real-time PCR according to the manufacturer's instructions. The results were interpreted automatically, and the analyzed data were presented using Bioneer's software (ExiStation™ and ExiDiagnosis). A positive result entails that the Ct value of the sample was below the cut-off Ct value. A negative result indicated the absence of infection confirmed by amplification of the internal control.

BIONEER's nucleic acid extraction eluted 400 µL of the clinical sample in a final volume of 100 µL using an automated extraction protocol on ExiStation™ and ExiPrep™ 48 Viral DNA/RNA Kit (Bioneer). The ExiStation™ system consisted of ExiPrep™48Dx (Bioneer) for RNA extraction and Exicycler™96 for real-time RT-PCR.

We used 50 µL aliquots for the AccuPower® RV1 Real-Time RT-PCR Kit analysis and 10 µL aliquots for the AccuPower® RV1 Multiplex Kit analysis. The AccuPower® RV1 Real-Time RT-PCR Kit was optimized for ExiStation™ and the AccuPower® RV1 Multiplex Kit was optimized for Exicycler™96. To determine clinical sensitivity and specificity, we compared the results of AccuPower® kits with those of the Allplex™ 2019-nCoV assay and the Standard M nCoV Real-Time Detection Kit for SARS-CoV-2 and those of the Allplex™ Respiratory Panel 1 for influenza viruses.

3. Sequencing

The samples that exhibited discrepancies in their results were confirmed by sequencing. We extracted the total RNA from the samples according to the manufacturer's instructions using the ExiStation™ and ExiPrep™ 48 Viral DNA/RNA Kit. We referred to the WHO protocol for the PCR conditions and primer sequences used for amplification [9]. Primer sequences for influenza were as follows: influenza type A M30F2/08: 5'-ATG AGY CTT YTA ACC GAG GTC GAA ACG-3' and influenza type A M264R3/08: 5'-TGG ACA AAN CGT CTA CGC TGC AG-3' for the M gene; influenza type B Victoria lineage Bvf224: 5'-ACA TAC CCT CGG CAA GAG

TTT C-3'; influenza type B Victoria lineage Bvr507: 5'-TGC TGT TTT GTT GTC GTT TT-3'; influenza type B Yamagata lineage BYf226: 5'-ACA CCT TCT GCG AAA GCT TCA-3'; and influenza type B Yamagata lineage BYf613: 5'-CAT AGA GGT TCT TCA TTT GGG TT-3' for the *HA* gene. Sequencing was performed using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

4. Analytical sensitivity and specificity

We determined the analytical sensitivity of SARS-CoV-2 using 3-fold serially diluted virus samples. For sputum, 900, 300, 100, 33.3, 11.1, and 3.7 copies/reaction, and for swabs, 300, 100, 33.3, 11.1, 3.7, and 1.23 copies/reaction, were tested in 72 replicates for six concentrations. Similarly, influenza A and B were diluted 3-fold from 600 copies/reaction to 0.27 copies/reaction and tested in 72 replicates for six concentrations.

SARS-CoV-2 was obtained from SeraCare Life Sciences (Milford, MA, USA) and influenza viruses from Zeptomatrix (Buffalo, NY, USA). Protocols for analytical sensitivity followed the Clinical and Laboratory Standards Institute guideline EP17-A2 [10]. Analytical sensitivity was estimated via probit regression analysis using R Studio (version 3.6.1).

The analytical specificities of the AccuPower® kits were tested using 40 different pathogens. DNA or RNA was extracted from each reference panel and assayed using the same protocols used for clinical samples.

5. Statistical analysis

We assessed significant differences between the two methods using 2 × 2 contingency tables and the VassarStats website (<http://vassarstats.net/>) and performed agreement statistics (kappa calculation) to compare the detection sensitivity of the AccuPower®

kits and the comparative methods. Clinical sensitivity, specificity, and kappa statistics were reported with 95% confidence intervals (CIs).

RESULTS

1. Comparison of the AccuPower® Kits and the confirmation results

Out of the 760 samples analyzed for SARS-CoV-2, 384 (190 sputum and 194 swab samples) were found to be positive, and 376 (188 sputum and 188 swab samples) were negative. Out of the samples tested for influenza, 81 were positive, and 95 were negative (Table 1).

For SARS-CoV-2, the evaluation was conducted at three institutions. The Green Cross Laboratory results were confirmed using the Allplex™ 2019-nCoV Assay and Seoul National University Hospital and Boramae Medical Center results using the STANDARD M nCoV Real-Time Detection Kit. The SARS-CoV-2 results analyzed with AccuPower® RV1 Kits were compared with the confirmed results from each institution (Table 2). Both the positive and negative agreement between the results from AccuPower® RV1 kits and the confirmation results for SARS-CoV-2 was 100%. The kappa values of the two methods were 1.0. Both the positive and negative agreement values of influenza A and influenza B between the results from AccuPower® RV1 Real-Time RT-PCR Kit and Allplex™ Respiratory Panel 1 were 100%. However, the positive and negative agreement between influenza A and B between the results from AccuPower® RV1 Multiplex Kit and Allplex™ Respiratory Panel 1 was 98.77%. The kappa value was 0.99.

The sequencing results for the discordant samples was positive. However, upon re-testing the samples with the comparative reagent (Allplex™ Respiratory Panel 1) and the AccuPower® RV1

Table 1. Comparison of the results of the AccuPower® RV1 Real-Time RT-PCR Kit and the confirmation tests for the detection of SARS-CoV-2 and influenza viruses

Virus	Specimen type	Agreement					
		Positive			Negative		
		%	No.	95% CI	%	No.	95% CI
SARS-CoV-2	Sputum	100	190/190	98.02–100	100	188/188	98.00–100
	Swab	100	194/194	98.06–100	100	188/188	98.00–100
Influenza A	Swab	100	81/81	95.47–100	100	81/81	95.47–100
Influenza B	Swab	100	81/81	95.47–100	100	95/95	96.11–100

Abbreviation: CI, confidence interval.

Table 2. Comparison of AccuPower® RV1 Real-Time RT-PCR Kit, AccuPower® RV1 Multiplex Kit and reference method

Virus	Specimen type	Agreement									
		No.		AccuPower® RV1 Real-Time RT-PCR Kit			No.		AccuPower® RV1 Multiplex Kit		
		Positive	Negative	Sensitivity (95% CI)	Specificity (95% CI)	Kappa (95% CI)	Positive	Negative	Sensitivity (95% CI)	Specificity (95% CI)	Kappa (95% CI)
SARS-CoV-2	Sputum	190/190	188/188	100 (98.02–100)	100 (98.00–100)	1 (0.99–1.00)	190/190	188/188	100 (98.02–100)	100 (98.00–100)	1 (0.99–1.00)
	Swab	194/194	188/188	100 (98.06–100)	100 (98.00–100)	1 (0.99–1.00)	194/194	188/188	100 (98.06–100)	100 (98.00–100)	1 (0.99–1.00)
Influenza A	Swab	80/81	81/81	100 (95.47–100)	100 (95.47–100)	1 (0.98–1.00)	80/81	81/81	98.77 (93.33–99.78)	100 (95.47–100)	0.99 (0.97–1.00)
Influenza B	Swab	81/81	95/95	100 (95.47–100)	100 (96.11–100)	1 (0.98–1.00)	80/81	95/95	98.77 (93.33–99.78)	100 (96.11–100)	0.99 (0.99–1.00)

Abbreviation: CI, confidence interval.

Real-Time RT-PCR Kit, the Ct values were detected near the cut-off in both kits. Therefore, the samples were low titer samples, and the discrepancy was attributed to the difference in the minimum nucleic acid concentration needed for use in the two tests.

2. Analytical sensitivity and specificity of the AccuPower® RV1 Kits

The analytical sensitivities of the AccuPower® RV1 Real-Time RT-PCR Kit were 64.57 copies/reaction (95% CI: 42.66–97.72), 56.23 copies/reaction (95% CI: 37.15–87.10), 38.90 copies/reaction (95% CI: 26.92–56.23), 37.15 copies/reaction (95% CI: 26.92–50.12), and 275.4 copies/reaction (95% CI: 19.05–39.81) for influenza A-H1N1 seasonal, H1N1 pdm, H3N2, influenza B-Yamagata, and influenza B-Victoria, respectively. The analytical sensitivities for the E gene and the SARS-CoV-2-specific gene (*RdRp* gene and *N* gene) were 107.15 copies/reaction (95% CI: 81.28–144.54) and 109.65 copies/reaction (95% CI: 79.43–147.91), respectively, with the sputum samples and 42.66 copies/reaction (95% CI: 32.36–54.95) and 26.92 copies/reaction (95% CI: 19.50–36.31), respectively, with the swab samples.

The analytical sensitivities of the AccuPower® RV1 Multiplex Kits were 47.86 copies/reaction (95% CI: 34.67–66.07), 37.15 copies/reaction (95% CI: 26.30–52.48), 33.11 copies/reaction (95% CI: 22.91–46.77), 33.88 copies/reaction (95% CI: 25.12–45.71), and 26.92 copies/reaction (95% CI: 21.38–33.11) for influenza A-H1N1 seasonal, H1N1 pdm, H3N2, influenza B-Yamagata, and influenza B-Victoria, respectively. The analytical sensitivities for the E gene and the SARS-CoV-2-specific gene (*RdRp* gene and *N* gene) were 117.48 copies/reaction (95% CI: 91.20–154.88) and 107.15 copies/reaction (95% CI: 83.18–141.25), respectively, with the sputum

samples and 44.67 copies/reaction (95% CI: 33.88–60.26) and 34.67 copies/reaction (95% CI: 26.30–44.67), respectively, with the swab samples.

We tested 40 different viruses and bacterial reference strains using the protocols used for the clinical samples to assess the cross-reactivity results and the detection specificity of the AccuPower® kits (Table 3). The test results were negative (34 species), except for the specific target (related SARS-CoV-2, Influenza A, and Influenza B). No non-specific positive reactions were observed.

DISCUSSION

Data on SARS-CoV-2 and influenza virus co-infection are limited. Influenza infection was classified as one of the characteristics of SARS-CoV-2 positive patients, and a small number of positive cases were studied. However, many studies have reported a correlation between the likelihood of co-infection and the severity or mortality rate due to co-infection [11–13]. Therefore, co-infection of SARS-CoV-2 and influenza viruses can pose serious risks. We can prepare for potential pandemics due to such co-infections by routinely testing for respiratory viruses other than SARS-CoV-2.

The AccuPower® RV1 Real-Time RT-PCR Kit contains dried primary materials required for PCR in one tube optimized for the *ExiStation*™ series. *ExiStation*™ can rapidly extract and analyze nucleic acid using an exclusive software. This minimizes the hands-on time after sample processing and any human error that may occur during the preparation of the reaction mixture. The test was completed within 2 hours and 30 minutes. Additionally, the AccuPower® RV1 Multiplex Kit is compatible with the

Table 3. Analytical specificities of the AccuPower® RV Real-Time RT-PCR Kit and the AccuPower® RV1 Multiplex Kit

No.	Pathogen	Strain	Result
1	SARS-CoV-2	Seracare (0505-0159)	Positive 1*
2	Human Influenza virus A H1N1 (seasonal)	KBPV VR-33	Positive 2 [†] ,
3	Human Influenza virus A H3N2 (Wisconsin/67/05)	Zeptomatrix 0810252CFHI	Positive 2
4	Human Influenza virus A H1N1 pdm virus (Michigan/45/15)	Zeptomatrix 0810538CFHI	Positive 2
5	Human Influenza virus B (Yamagata/16/88)	Zeptomatrix 0810518CFHI	Positive 3 [†]
6	Human Influenza virus B (Texas/6/11)	Zeptomatrix 0810242CFHI	Positive 3
7	Human Coronavirus 229E	KBPV VR-9	Negative
8	Human Coronavirus NL63	Zeptomatrix 0810228CF	Negative
9	Human Coronavirus OC43	Zeptomatrix 0810024CF	Negative
10	Human Coronavirus OC43	KBPV VR-8	Negative
11	Coronavirus HKU1 (synthetic RNA)	ATCC 3262SD	Negative
12	SARS-Coronavirus (Purified RNA of SARS-Coronavirus strain Frankfurt 1)	EVAg 004N-02005	Negative
13	Parainfluenza virus 1	Zeptomatrix 0810014CFHI	Negative
14	Parainfluenza virus 2	Zeptomatrix 0810015CFHI	Negative
15	Parainfluenza virus 3	Zeptomatrix 0810016CFHI	Negative
16	Parainfluenza virus 4a	Zeptomatrix 0810060CFHI	Negative
17	Parainfluenza virus 4b	Zeptomatrix 0810060BCFHI	Negative
18	Human Respiratory syncytial virus A	KBPV VR-41	Negative
19	Human Respiratory syncytial virus B	KBPV VR-42	Negative
20	Human Rhinovirus 1 (type A)	KBPV VR-81	Negative
21	Human Rhinovirus 7 (type A)	KBPV VR-82	Negative
22	Human Rhinovirus 8 (type B)	KBPV VR-79	Negative
23	Human Rhinovirus 42 (type B)	KBPV VR-80	Negative
24	Human Metapneumovirus type 3 Type B1	Zeptomatrix 0810156CFHI	Negative
25	Human Metapneumovirus type 9 Type A1	Zeptomatrix 0810160CFHI	Negative
26	Human Parechovirus 1	Zeptomatrix 0810145CF	Negative
27	Human Parechovirus 2	Zeptomatrix 0810146CF	Negative
28	Human Parechovirus 3	Zeptomatrix 0810147CF	Negative
29	Human Adenovirus type 03 (type B)	KBPV VR-62	Negative
30	Human Adenovirus type 02 (type C)	KBPV VR-58	Negative
31	Human Bocavirus (synthetic DNA)	ATCC 3251SD	Negative
32	Enterovirus 70	KBPV VR-55	Negative
33	Coxsackievirus B5	KBPV VR-17	Negative
34	Echovirus 25	KBPV VR-24	Negative
35	MERS-CoV	Seracare 0505-0002	Negative
36	<i>Chlamydia pneumoniae</i>	ATCC 53592	Negative
37	<i>Haemophilus influenzae</i>	ATCC 31441	Negative
38	<i>Legionella pneumophila</i>	ATCC 33152	Negative
39	<i>Streptococcus pneumoniae</i>	ATCC 49619	Negative
40	<i>Bordetella pertussis</i>	ATCC 12742	Negative

*Detects only SARS-CoV-2; [†]Detects only Influenza A; [‡]Detects only Influenza B.

Abbreviations (strain): KBPV, Korea Bank for Pathogenic Viruses; EVAg, European Virus Archive-Global.

ABI7500 instrument (Applied Biosystems, Foster City, CA), CFX96 instrument (Bio-Rad, Hercules, CA), and the *Exicycler*™.

We evaluated the cross-reactivity with 40 respiratory pathogens, including SARS-CoV-2, influenza A, and influenza B, and confirmed that there was no cross-reactivity to pathogens with similar symptoms.

We evaluated the limit of detection (LoD) in the sputum and

nasopharyngeal/oropharyngeal swabs. The LoD in sputum was approximately 100 copies/test, and that in the swabs was approximately 40 copies/test in both kits. The rate of positivity is higher in sputum samples than that in the swabs and is less affected by the onset of symptoms or the time of collection [14]. However, sputum samples are usually viscous and contain many PCR and extraction inhibitors; therefore, we recommend performing a pre-

treatment process for nucleic acid extraction for real-time PCR. Homogenization of sputum with proteinase K, dithiothreitol, or N-acetyl-L-cysteine (NALC) can increase the sensitivity of the test, and hence, the rate of positivity [15, 16].

We confirmed the clinical sensitivity and specificity by comparing the confirmation results of the AccuPower® RV1 Real-Time RT-PCR Kit and the AccuPower® RV1 Multiplex Kit using residual clinical samples collected from three institutions. The sensitivity and specificity were 100%, which is consistent with the confirmation results for both positive and negative samples of SARS-CoV-2. One influenza A sample and some influenza B samples displayed a discrepant result in the AccuPower® RV1 Multiplex Kit. Sensitivity and specificity were 98.77%. The kappa values for all targets were ≥ 0.99 and were considered clinically valid when compared with the results of the confirmation tests.

A limitation of our study is that we did not include asymptomatic, pre-symptomatic, and co-infection cases, as only residual samples were used. Further studies, including these cases, need to be conducted.

In conclusion, the AccuPower® RV1 Kits produced results comparable to the Allplex™ and Standard M Kit. The AccuPower® RV1 Kits showed a higher concordance with the results of the comparative methods. Thus, the AccuPower® RV1 Kits are a valuable tool to detect influenza A, B, SARS-CoV-2, and their co-infections in clinical laboratories.

요 약

배경: AccuPower® RV1 Real-Time RT-PCR 키트(Bioneer, Korea)와 AccuPower® RV1 Multiplex 키트(Bioneer)는 원스텝 실시간 역전사 중합효소연쇄반응법으로 SARS-CoV-2, 인플루엔자 A, 인플루엔자 B의 핵산을 검출한다.

방법: 우리는 1,098개의 임상검체를 이용하여 AccuPower® RV1 Real-Time RT-PCR 키트와 AccuPower® RV1 Multiplex 키트를 평가하였다. SARS-CoV-2의 존재는 Allplex™ 2019-nCoV Assay (Seegene, Korea)와 Standard M nCoV Real-Time Detection Kit (SD Biosensor, Korea)를 이용하여 재확인하였다. 인플루엔자 바이러스는 Allplex™ Respiratory Panel 1 (Seegene)을 이용하여 재확인하였다.

결과: AccuPower® RV1 Real-Time RT-PCR 키트의 양성 및 음성 일치도는 100%였다. AccuPower® RV1 Multiplex 키트의 양성일치도는 SARS-CoV-2에 대해서는 100%였고 인플루엔자 A와 B에 대해서

는 98.77%였다. SARS-CoV-2와 인플루엔자 A, B에 대한 카파 값은 0.99를 넘었다. SARS-CoV-2는 객담과 비인두 및 구강인두 스왑 검체를 이용하여 평가하였다. 검체 종류에 따른 검출률의 차이는 없었다.

결론: AccuPower® RV1 Real-Time RT-PCR 키트와 AccuPower® RV1 Multiplex 키트는 임상적으로 사용되는 데 적합한 성능을 보여주었다.

Conflicts of Interest

None declared.

Acknowledgements

This research was supported by a grant from 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: HI20C2038). Some bioresources from the National Biobank of Korea and the Korea Disease Control and Prevention Agency (grant number: 2020-045) were used.

REFERENCES

1. Kong WH, Li Y, Peng MW, Kong DG, Yang XB, Wang L, et al. SARS-CoV-2 detection in patients with influenza-like illness. *Nat Microbiol* 2020;5:675-8.
2. Guo JJ, Yu YH, Ma XY, Liu YN, Fang Q, Qu P, et al. A multiple-center clinical evaluation of a new real-time reverse transcriptase PCR diagnostic kit for SARS-CoV-2. *Future Virol* 2020;10.2217/fvi-2020-0299.
3. Attwood LO, Francis MJ, Hamblin J, Korman TM, Druce J, Graham M. Clinical evaluation of AusDiagnostics SARS-CoV-2 multiplex tandem PCR assay. *J Clin Virol* 2020;128:104448.
4. Singer BD. COVID-19 and the next influenza season. *Sci Adv* 2020;6:eabd0086.
5. Bai L, Zhao Y, Dong J, Liang S, Guo M, Liu X, et al. Coinfection with influenza A virus enhances SARS-CoV-2 infectivity. *Cell Res* 2021;31:395-403.
6. Wu X, Cai Y, Huang X, Yu X, Zhao L, Wang F, et al. Co-infection with SARS-CoV-2 and influenza A virus in patient with pneumonia, China. *Emerg Infect Dis* 2020;26:1324-6.
7. Cuadrado-Payán E, Montagud-Marrahi E, Torres-Elorza M, Bodro M,

- Blasco M, Poch E, et al. SARS-CoV-2 and influenza virus co-infection. *Lancet* 2020;395:e84.
8. Chung HY, Jian MJ, Chang CK, Lin JC, Yeh KM, Chen CW, et al. 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* 2021;10:161-6.
9. Mancini F, Barbanti F, Scaturro M, Fontana S, Di Martino A, Marsili G, et al. Multiplex real-time reverse-transcription polymerase chain reaction assays for diagnostic testing of severe acute respiratory syndrome coronavirus 2 and seasonal influenza viruses: A challenge of the phase 3 pandemic setting. *J Infect Dis* 2021;223:765-74.
10. Clinical and Laboratory Standards Institute. Evaluation of detection capability for clinical laboratory measurement procedures; Approved guideline—Second edition, CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
11. Alosaimi B, Naeem A, Hamed ME, Alkadi HS, Alanazi T, Al Rehily SS, et al. Influenza co-infection associated with severity and mortality in COVID-19 patients. *Virol J* 2021;18:127.
12. D'Abramo A, Lepore L, Palazzolo C, Barreca F, Liuzzi G, Lalle E, et al. Acute respiratory distress syndrome due to SARS-CoV-2 and Influenza A co-infection in an Italian patient: Mini-review of the literature. *Int J Infect Dis* 2020;97:236-9.
13. Konala VM, Adapa S, Gayam V, Naramala S, Daggubati SR, Kammari CB, et al. Co-infection with Influenza A and COVID-19. *Eur J Case Rep Intern Med* 2020;7:001656.
14. Mohammadi A, Esmaeilzadeh E, Li Y, Bosch RJ, Li JZ. SARS-CoV-2 detection in different respiratory sites: A systematic review and meta-analysis. *EBioMedicine* 2020;59:102903.
15. Yu F, Qiu T, Zeng Y, Wang Y, Zheng S, Chen X, et al. Comparative evaluation of three preprocessing methods for extraction and detection of influenza A virus nucleic acids from sputum. *Front Med* 2018;5:56.
16. Lin C, Xiang J, Yan M, Li H, Huang S, Shen C. Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov-2)-infected pneumonia (COVID-19). *Clin Chem Lab Med* 2020;58:1089-94.