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ORIGINAL ARTICLE

Respiratory Viruses Co-detection on a Multiplex RT-PCR Panel: a Comparative Clinical Study

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SUMMARY

Background: **The FilmArray Respiratory Panel RP 2.1 plus (FilmArray RP) is a point-of-care syndromic panel for respiratory pathogens. Although highly valuable in the clinical settings, the co-detection of pathogens in FilmArray RP may confound result interpretation.**
Methods: **Nasopharyngeal swab specimens collected from patients with respiratory symptoms were analyzed by comparing co-detection results from FilmArray RP with those of Allplex Respiratory Panels (Allplex RP: PowerChek for SARS-CoV-2).**
Results: **Out of 765 FilmArray RP tests, 143 (18.7%) showed co-detections (two: 122 (85.3%), three: 18 (12.6%), four: 2 (1.4%), and five viruses: 1 (0.7%). The most frequent co-detection was human rhinovirus/enterovirus (HRV/HEV) with respiratory syncytial virus (RSV) (22.3%, 32/143). The overall discordance rate between FilmArray RP and other tests was 32.9%. Notably, discordance in detecting adenovirus (AdV) was significant, with cases detected by FilmArray often not appearing in Allplex RP.**
Conclusions: **Discordances were varied by virus combination. It is advisable to perform additional confirmatory testing based on clinical relevance.**
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INTRODUCTION

Respiratory infections constitute a major factor in contributing to high mortality rates in hospitals and communities [1-6]. A rapid diagnosis and treatment significantly impact patient´s outcomes. Therefore, the use of multiplex real-time PCR systems with point-of-care syndromic panels, which allow all processes to be conducted using a single kit, is highly valuable in clinical settings [7,8]. The FilmArray Respiratory Panel RP2.1 plus (FilmArray RP; BioFire Diagnostics Marcy l'Etoile, France) is a point-of-care syndromic panel that targets respiratory pathogens. It can detect 19 viruses and four bacteria that can cause respiratory infections [9]. For FilmArray RP, there have been reports of more

than one pathogen being detected in a sample, which may confound result interpretation [10]. To date, research has primarily focused on the detection of SARS-CoV-2 alongside other pathogens, with limited literatures on the co-detection of pathogens. Therefore, we aimed to compare the co-detection results found on FilmArray RP with the results of other multiplex-PCR tests.

MATERIALS AND METHODS

Sample collection

Nasopharyngeal swabs (NPS) were collected from symptomatic patients who visited the 500-bed general hospital in Korea between March 27 and May 26, 2023. The samples were tested using FilmArray RP, and those samples wherein two or more viruses were detected were collected for further analysis. This study was approved by the Institutional Review Board (Approval no.: CNUSH 2023-11-005). Informed consent from patients was not obtained as this was a retrospective study using residual samples.

FilmArray RP

The FilmArray RP is a fully automated multiplex PCR system. In this method, a 300 μL sample from an NPS is combined with 3 mL of viral transport medium. This mixture is then placed into the BioFire pouch and loaded into the FilmArray RP instrument. Subsequently, the system performs nucleic acid extraction and nested multiplex PCR in a single step. The assay automatically generates and interprets the melting curves, classifying results as ‘detected’ or ‘not detected’ based on the manufacturer’s guidelines. Human coronavirus (HCoV) HKU1, Middle East respiratory syndrome coronavirus, and four bacteria, which are not included in the Allplex RP panel, were excluded from the analysis.

Allplex Respiratory Panels (Allplex RP) 1, 2, 3

Allplex RP 1, 2, 3 (Seegene Inc., Seoul, Korea) utilizes a one-step RT-PCR approach based on the multiple detection temperature technology. The assay is designed to identify 19 different viruses from one NPS sample, divided into three panels. Testing was conducted according to the manufacturer's guidelines. Analytical interpretation of the results was automated via Seegene Viewer (Seegene Inc.). Human bocavirus, which is not included in the FilmArray RP panel, was excluded from the analysis.

RT-PCR for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

The NPS samples that tested positive for SARS-CoV-2, using the FilmArray RP, were analyzed by using the PowerChek SARS-CoV-2, Influenza A & B Multiplex Real-time PCR Kit (Kogene Biotech, Seoul, Korea). The criterion for a positive SARS-CoV-2 test was established as a fluorescence curve surpassing the threshold within 38 cycles, and confirmation required positive results for both E and ORF1ab target genes.

RESULTS

During the study period, 765 FilmArray RP were conducted on patients with respiratory symptoms. Among the total cases, 18.7% (143/765) showed presence of multiple viruses (Table 1). A total of 122 cases of two respiratory targets (85.3%), 18 of three respiratory targets (12.6%), two of four respiratory targets (1.4%), and one of five respiratory targets (0.7%) were detected.

The most frequently detected combination of multiple viruses was HRV/HEV and RSV (22.3%, 32/143), followed by the combination of AdV with HRV/HEV (18.2%, 26/143) and parainfluenza virus 3 (PIV3) with HRV/HEV (14.7%, 21/143). There were also several cases of three viruses detected, including five cases (3.5%) of the combination of AdV, PIV3, and HRV/HEV, three (2.1%) of the combination of AdV, metapneumovirus (MPV), and HRV/HEV, and three (2.1%) of the combination of AdV, HRV/HEV, and RSV. Notably, our study identified two instances (1.4%), wherein a combination of four different viruses was detected and a case (0.7%), wherein a combination of five different viruses was found.

When two or more viruses were detected in FilmArray RP, the overall discordance rate with Allplex RP (PowerChek for SARS-CoV-2) was 32.9% (47/143) (Table 2). More specifically, for cases identifying two viruses, this rate was 30.3% (37/122). When both AdV and PIV3 were detected in FilmArray RP, it showed the highest discordance rate (87.5%). There were seven (26.9%) discordances for co-detection of AdV and HRV/HEV, all of which were undetectable for AdV. Five (23.8%) discordances were found regarding co-detection of HRV/HEV and PIV3. The combination of HRV/HEV and RSV, which constituted the highest proportion of co-detected cases, had five discordances (15.6%).

When three viruses were detected in the FilmArray RP, the overall discordance rate with the Allplex RP was 50.0% (9/18). There were six cases where only one virus was not detected in the Allplex RP. Notably, AdV was not detected in five of the six cases.

In one case, five viruses were detected using the FilmArray RP: AdV, HCoV NL63, influenza virus A (FluA) H3, MPV, and HRV/HEV, while only MPV and HRV were detected using the Allplex RP.

The degree of concordance and discordance was analyzed for each virus (Figure 1). The most frequently detected virus was HRV/HEV (111 cases), but it showed only two discordances, resulting in a low discordance rate of 1.8%. AdV and RSV were the second most frequently detected viruses, each identified in 58 cases. Their discordance rates were 31.1% and 15.5%, respectively.

Table 1. Co-detection of respiratory viruses on FilmArray RP.

Number of viruses	Detected viruses	Number of cases (%)
2	HRV/HEV + RSV	32 (22.4%)
	AdV + HRV/HEV	26 (18.2%)
	PIV3 + HRV/HEV	21 (14.6%)
	AdV + PIV3	8 (5.6%)
	AdV + RSV	6 (4.2%)
	PIV3 + RSV	4 (2.8%)
	MPV + HRV/HEV	4 (2.8%)
	FluA H3 + HRV/HEV	4 (2.8%)
	FluA H3 + RSV	3 (2.1%)
	HRV/HEV + SARS-CoV-2	2 (1.4%)
	FluA H1-2009 + HRV/HEV	2 (1.4%)
	AdV + FluA H1-2009	1 (0.7%)
	AdV + FluA H3	1 (0.7%)
	AdV + MPV	1 (0.7%)
	HCoV 229E + PIV4	1 (0.7%)
	HCoV 229E + RSV	1 (0.7%)
	HCoV OC43 + PIV3	1 (0.7%)
	HCoV OC43 + RSV	1 (0.7%)
	FluA H1-2009 + MPV	1 (0.7%)
	PIV3 + SARS-CoV-2	1 (0.7%)
	PIV4 + RSV	1 (0.7%)
3	AdV + PIV3 + HRV/HEV	5 (3.5%)
	AdV + MPV + HRV/HEV	3 (2.1%)
	AdV + HRV/HEV + RSV	3 (2.1%)
	AdV + PIV2 + HRV/HEV	1 (0.7%)
	AdV + PIV3 + RSV	1 (0.7%)
	HCoV NL63 + HRV/HEV + RSV	1 (0.7%)
	FluA H1-2009 + PIV4 + HRV/HEV	1 (0.7%)
	FluA H3 + HRV/HEV + RSV	1 (0.7%)
	PIV3 + HRV/HEV + RSV	1 (0.7%)
	HRV/HEV + RSV + SARS-CoV-2	1 (0.7%)
4	AdV + PIV3 + HRV/HEV + RSV	1 (0.7%)
	HCoV OC43 + PIV3 + HRV/HEV + RSV	1 (0.7%)
5	AdV + HCoV NL63 + FluA H3 + MPV + HRV/HEV	1 (0.7%)

AdV - adenovirus, HCoV - human coronavirus, MPV - metapneumovirus, FluA - influenza virus A, PIV - parainfluenza virus, HRV/HEV - human rhinovirus/enterovirus, RSV - respiratory syncytial virus, SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2.

DISCUSSION

In this study, we compared the co-detection results of FilmArray RP with the results of Allplex RP 1, 2, 3 and PowerChek (for SARS-CoV-2). While FilmArray RP has the advantage of rapidly detecting a wide range of respiratory pathogens simultaneously, this study found a significant number of discordances (32.9%) in cases of co-detection. Clinically common viruses were often detected simultaneously, and the concordance rate was high, while the number of discordances for specific viruses was also high.

Through this study, we found a high co-detection rate of HRV/HEV with RSV, AdV with HRV/HEV, and PIV3

Table 2. Comparison of co-detection results from FilmArray RP with those of Allplex RP (PowerChek for SARS-CoV-2).

Number of viruses	Detected viruses	Number of discordant cases (%)	Results of other tests (number of cases)
2	AdV + PIV3	7 (87.5%)	Not detected(1), AdV(3), PIV3(1), HRV + PIV3(1), AdV + HRV + PIV3(1)
	AdV + HRV/HEV	7 (26.9%)	HRV(5), HEV + HRV(2)
	PIV3 + HRV/HEV	5 (23.8%)	HEV(1), HRV(2), AdV + HRV + PIV3(1), PIV3 + HRV + RSV B(1)
	HRV/HEV + RSV	5 (15.6%)	RSV A(1), HRV(3), RSV A+HRV+PIV3+AdV(1)
	FluA H3 + RSV	3 (100%)	FluA H3(2), RSV A(1)
	PIV3 + RSV	2 (50%)	RSV B(1), PIV3(1)
	MPV + HRV/HEV	2 (50%)	HRV(1), AdV + HRV + MPV(1)
	AdV + RSV	2 (33.3%)	AdV(1), RSV B(1)
	AdV + FluA H3	1 (100%)	FluA H3 + HRV(1)
	AdV + MPV	1 (100%)	AdV (1)
	HCoV OC43 + RSV	1 (100%)	RSV B(1)
	PIV3 + SARS-CoV-2	1 (100%)	SARS-CoV-2(1)
3	AdV + PIV3 + HRV/HEV	2 (40%)	PIV3 + HRV(2)
	AdV + PIV2 + HRV/HEV	1 (100%)	PIV2 + HRV(1)
	AdV + PIV3 + RSV	1 (100%)	PIV3 + RSV B(1)
	FluA H1-2009 + PIV4 + HRV/HEV	1 (100%)	FluA H1-2009 + HRV(1)
	FluA H3 + HRV/HEV + RSV	1 (100%)	FluA H3(1)
	PIV3 + HRV/HEV + RSV	1 (100%)	HRV(1)
	HRV/HEV + RSV + SARS-CoV-2	1 (100%)	HRV + RSV B(1)
	AdV + MPV + HRV/HEV	1 (33.3%)	MPV + HRV + HEV(1)
5	AdV + HCoV NL63 + FluA H3 + MPV + HRV/HEV	1 (100%)	MPV + HRV(1)

AdV - adenovirus, HCoV - human coronavirus, MPV - metapneumovirus, FluA - influenza virus A, PIV - parainfluenza virus, HRV/HEV - human rhinovirus/enterovirus, RSV - respiratory syncytial virus, SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2.

with HRV/HEV. These viruses have been reported to be highly prevalent in other studies, indicating that each pathogen is a major cause of respiratory tract infections and is likely to be co-infected [10-12]. In particular, HRV/HEV was detected at the highest frequency in co-detections by FilmArray RP, with a detection count of 111. In other domestic study comparing FilmArray RP and Allplex RP, including single detections, HRV/HEV was the most frequently detected virus [10]. Despite the high number of detections, there were only two discordances (1.8%). Out of the 109 concordant HRV/HEV cases, 105 (96.8%) had HRV observed on the Allplex RP, 11 (10.1%) had HEV, and of these, 7 (6.4%) had both HRV and HEV. The two discordances were cases wherein neither HRV nor HEV was observed on the Allplex RP.

Notably, we observed a high discordance rate for AdV in this study. In various combinations, AdV detected by the FilmArray was missing from the Allplex results, as shown in Table 2. Also, Figure 1 shows that AdV had the highest discordance rate (31.0%), excluding viruses with less than 10 detections. Lade et al. reported a better detection of AdV on FilmArray RP than on Allplex RP. We note that this may be due to the low concentration of AdV in samples or the high sensitivity of FilmArray RP to AdV [9,10].

In this study, four viruses were detected simultaneously in two cases: the combination of PIV3, HRV/HEV, and RSV, with each case additionally containing either AdV or HCoV OC43. Both results were in agreement with Allplex RP. This suggests that a large number of simultaneously detected isolates should not be considered false positives without further testing.

This study had some limitations. First, this study was conducted at a single institution, which limits the generalizability of the results. Second, because they are not included in the Allplex RP, HCoV HKU1 and the four bacteria detected by the FilmArray RP were not evaluat-

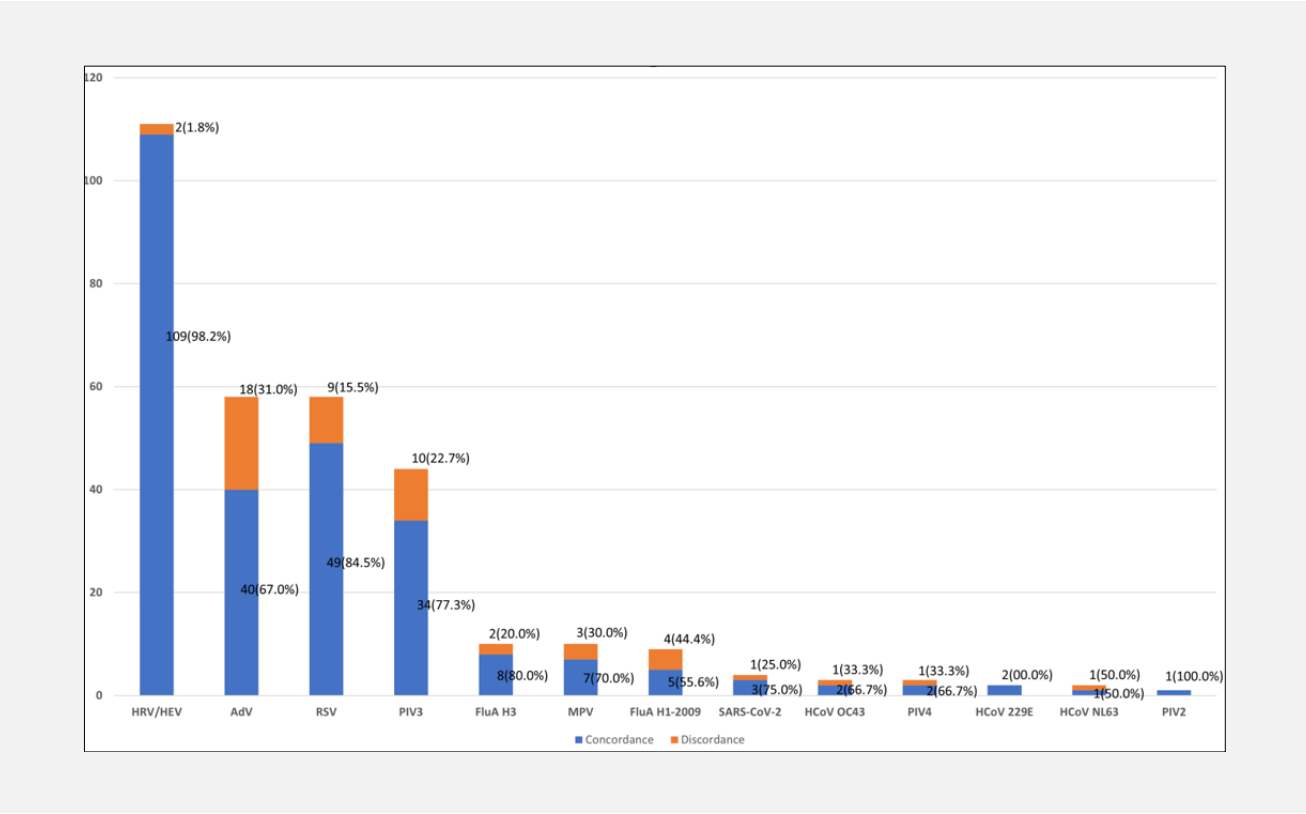

Figure 1. Frequency of detection and discordances for each virus.

Abbreviations: AdV - adenovirus, HCoV - human coronavirus, MPV - metapneumovirus, FluA - influenza virus A, PIV - parainfluenza virus, HRV/HEV - human rhinovirus/enterovirus, RSV - respiratory syncytial virus, SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2.

ed. Third, our comparison was limited to two specific tests without considering other confirmatory testing methods, such as single PCR or sequencing. Fourth, when faced with discordances in positive findings between the Allplex RP and the FilmArray RP, we could not provide a definitive conclusion on which assay presents the accurate result. This uncertainty leaves room for further investigation into the accuracy of these tests. Finally, our hospital was actively conducting other independent testings for SARS-CoV-2 during the study period, which precluded a proper evaluation. There are several prior studies on FilmArray RP that focused on SARS-CoV-2 detection [13-15]. According to these studies, SARS-CoV-2 can be detected alongside other common respiratory viruses, and discordances can be observed with other testing methods, suggesting that further testing with other methods is required.

In conclusion, we demonstrated concordances and discordances in the detection of multiple viral analytes between FilmArray RP 2.1 Plus and Allplex RP 1, 2, and 3. The overall discordance rate was 32.9%, but discordances were varied by virus combination. It is advisable to perform additional confirmatory testing based on clinical relevance.

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Declaration of Interest:
The authors declare that there is no conflict of interest.

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