Journal of Microbiology, Immunology and Infection (2021) 54, 518–521

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Short Communication

Performance evaluation of EuDx™ ufPCR Flu & RSV detection kit for detection of influenza A/B and respiratory syncytial virus

Yong Kwan Lim [a], Oh Joo Kweon [a], Hye Ryoun Kim [a], Tae-Hyoung Kim [b], Ah Ra Cho [c], Mi-Kyung Lee [a,*]

[a] *Department of Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Republic of Korea*
[b] *Department of Urology, Chung-Ang University College of Medicine, Seoul, Republic of Korea*
[c] *Seoul Clinical Laboratories, Gyeonggi-do, Republic of Korea*

Received 18 December 2019; received in revised form 12 June 2020; accepted 24 June 2020
Available online 27 June 2020

KEYWORDS
PCR;
Diagnostic tests;
Influenza virus;
Respiratory syncytial virus;
Evaluation

Abstract EuDx™ ufPCR Flu & RSV Detection Kit (EUDIPIA, Chungcheongbuk-do, Republic of Korea) is a recently developed molecular assay for simultaneously detecting influenza A/B and respiratory syncytial virus (RSV). We evaluated this assay in a clinical setting and demonstrated its excellent performance for diagnosing influenza A/B and RSV infections.

Introduction

Acute respiratory tract infection is one of the most common illnesses worldwide, regardless of age or sex.[1] Especially, viral respiratory infections are unfavorably known for their ability to rapidly spread within communities. Influenza annually causes respiratory tract infections in 5–15% of the global population and severe illness in about 3–5 million people.[2] Respiratory syncytial virus (RSV) is a common cause of childhood acute respiratory infection, causing almost 33 million episodes yearly.[3]

Early diagnosis of these viruses is essential, especially in hospitalized patients, for shifting from empirical therapy to targeted therapy and for preventing viral transmission.[4] For this reason, there is a need for better diagnostic tests including better methods for the rapid detection of infectious agents in clinical laboratories.[2] Generally, rapid antigen kits are frequently used in the clinical setting because these tests yield results within about 30 min. However, owing to the relatively high number of false-negative results in rapid tests, a more sensitive test is desirable.[5] Real-time polymerase chain reaction (RT-PCR) may be the most

* Corresponding author. 102 Heukseok-ro, Dongjak-gu, Seoul, 06973, Republic of Korea. Fax: +82 2 6298 8630.
E-mail address: cpworld@cau.ac.kr (M.-K. Lee).

https://doi.org/10.1016/j.jmii.2020.06.012

commonly preferred confirmatory method because it has higher sensitivity and specificity than rapid antigen tests.[6]

Recently, EuDx™ ufPCR Flu & RSV Detection Kit (EuDx ufPCR Flu & RSV; EUDIPIA, Chungcheongbuk-do, Republic of Korea) has been developed as a real-time ultra-fast PCR (ufPCR) assay for respiratory viruses that can simultaneously detect influenza A and B viruses and RSV within 30 min. In this study, to evaluate the analytical performance of EuDx ufPCR Flu & RSV, we compared the results of 316 respiratory samples to those of two conventional multiplex RT-PCR assays: AdvanSure Respiratory virus real-time PCR kit (LG Life Sciences, Seoul, Republic of Korea) and Allplex™ Respiratory Panel 1 (Seegene, Seoul, Republic of Korea). Additionally, we evaluated the detection sensitivity and cross-reactivity of this assay.

Methods

Clinical samples

A total of 316 respiratory specimens (251 from pediatric patients and 65 from adult patients) were obtained to evaluate the performance of EuDx ufPCR Flu & RSV. Of them, 60, 116, and 80 specimens tested positive for influenza A, influenza B, and RSV when assessed using AdvanSure Respiratory virus real-time PCR kit, respectively. Further, 60 respiratory virus-negative samples were selected. The specimens included nasal swabs ($n = 219$), throat swabs ($n = 63$), nasal aspirates ($n = 28$), and sputum ($n = 6$). The specimens were stored at −70 °C after nucleic acid extraction and PCR assays. The protocol was approved by the institutional review board of Chung-Ang University Hospital (Seoul, Republic of Korea; approval no. 1861-005-329).

EuDx™ ufPCR Flu & RSV detection kit

EuDx ufPCR Flu & RSV is based on the ufPCR system for influenza A/B and RSV in respiratory specimens. This system adopts a microfluidic polymer chip, which performs rapid amplification of genomic templates and real-time qualitative and quantitative analyses. Each targeted virus is amplified in a channel on a Rapi:chip™ PCR chip (Fig. 1), and EuDx™ GENECHECKER® ufPCR System (EUDIPIA) detects the fluorescence generated by the binding of EvaGreen™ dye to the amplified products in real time. The ufPCR process takes only 30 min for reporting the results, and its portable characteristic allows point-of-care tests.

Evaluation protocol

Nucleic acid extraction was performed using the NucliSens easyMAG instrument (bioMerieux, Marcy l'Etoile, France), according to the manufacturer's instructions. For comparison with other commercially available kits, all samples were tested with EuDx ufPCR Flu & RSV, AdvanSure Respiratory virus real-time PCR kit (LG Life Sciences), and Allplex™ Respiratory Panel 1 (Seegene). Positive results in two or more kits were considered to be a consensus of positive results. Conversely, if two or more tests were negative, they were considered to be a negative consensus. Thereafter, the sensitivity and specificity for influenza A, influenza B, and RSV were calculated. For assessing the reliability of agreement among the three respiratory virus assays, Fleiss' kappa coefficients were additionally calculated.

For evaluating the detection sensitivities for influenza A/B and RSV, we performed a precision analysis using different virus titers. Precision analysis was performed by repeating the test with 4 concentrations of the test sample 6 times a day for 10 days (1.0×10^4, 1.0×10^3, 1.0×10^2 and 1.0×10^1 copies per run). To evaluate the cross-reactivity with other pathogens, we spiked the influenza A/B- and RSV-negative specimens with 22 other pathogens (Supplement 1). Then, we tested these specimens thrice with EuDx ufPCR Flu & RSV.

Statistics

The sensitivity, specificity, and Fleiss' kappa coefficient were calculated using Microsoft Office Excel 2010 (Microsoft Co., Redmond, WA, USA) and R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria) according to Clinical and Laboratory Standards Institute guideline EP12-A2.[7] The kappa coefficient was assessed according to following criteria: 0.81—1.00 for almost perfect agreement, 0.61—0.80 for substantial agreement, 0.41—0.60 for moderate agreement, 0.21—0.40 for fair agreement, 0.00—0.20 for slight agreement, and <0.00 for poor agreement.

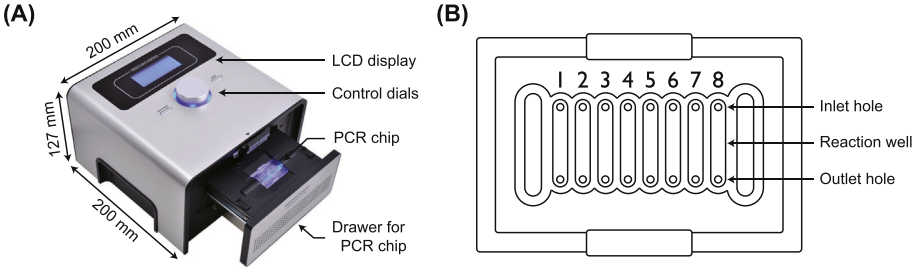

Figure 1. EuDx™ GENECHECKER® ultra-fast real-time polymerase chain reaction (PCR) system (A) and Rapi:chip™ PCR chip (B). Samples are loaded into the inlet hole on the PCR chip, and PCR reactions are completed within 30 min. Lane 1, influenza A. Lanes 2—4, influenza A subtypes pdm09, H3, and H5. Lane 5, influenza B. Lane 6, respiratory syncytial virus. Lanes 7 and 8, negative and internal controls.

Table 1 Diagnostic performances for detecting respiratory pathogens.

	TP	FP	FN	TN	Sensitivity	Specificity	Fleiss' kappa
Influenza A							
EuDx ufPCR Flu & RSV	58	0	1	257	98.3 (90.9–100)	100 (98.6–100)	0.986 (0.830–1)
LG AdvanSure	59	1	0	256	100 (93.9–100)	99.6 (97.9–100)	
Seegene Allplex	59	0	0	257	100 (93.9–100)	100 (98.6–100)	
Influenza B							
EuDx ufPCR Flu & RSV	110	0	4	202	96.5 (91.3–99.0)	100 (98.2–100)	0.972 (0.799–1)
LG AdvanSure	114	2	0	200	100 (96.8–100)	99.0 (96.5–99.9)	
Seegene Allplex	114	0	0	202	100 (96.8–100)	100 (98.2–100)	
RSV							
EuDx ufPCR Flu & RSV	77	0	1	238	98.7 (93.1–100)	100 (98.5–100)	0.977 (0.817–1)
LG AdvanSure	78	2	0	236	100 (95.4–100)	99.2 (97.0–99.9)	
Seegene Allplex	78	1	0	237	100 (95.4–100)	99.6 (97.7–100)	

TP, true positive; FP, false positive; FN, false negative; TN, true negative; RSV, respiratory syncytial virus.

Results

EuDx ufPCR Flu & RSV and two other conventional multiplex RT-PCR assays were simultaneously performed in 316 respiratory specimens. According to the results of the three assays, a consensus of positive results for influenza A, influenza B, and RSV was achieved in 59, 114, and 78 specimens, respectively. In the comparison of EuDx ufPCR Flu & RSV with the consensus results, its sensitivity was 98.3% for influenza A, 96.5% for influenza B, and 98.7% for RSV, and its specificity was 100% for all three viruses (Table 1). When assessing the agreement among the three assays, the Fleiss' kappa coefficients were 0.986, 0.972, and 0.977 for influenza A, influenza B, and RSV, respectively, and these results were consistent with an almost perfect agreement.

On evaluating the detection sensitivity of EuDx ufPCR Flu & RSV, we found that the detection rates for influenza A/B and RSV samples were at least 95% at copies per run (Supplement 2). In the cross-reactivity evaluation, pathogens other than the target pathogen were not amplified or detected in any sample. We concluded that EuDx ufPCR Flu & RSV had an excellent detection sensitivity for about 1.0×10^2 copies of influenza A/B and RSV and that it did not show cross-reactivity for any other pathogens.

Conclusion

Because there are various potential pathogens that may cause similar characteristics of respiratory tract infection, clinical symptoms are rarely sufficiently distinctive to specify the causative pathogen of respiratory illnesses.[8] Clinicians need to rely on laboratory confirmation of a clinically suspected pathogen to decide on the treatment plan and hospitalization of patients.[9] For rapid and accurate detection of respiratory pathogens, a wide variety of commercial PCR assays have been introduced. Recently, large syndromic panels that can simultaneously detect multiple respiratory viruses have gained large popularity among clinical laboratories. These assays are beneficial for making a timely treatment decision and for effective infection prevention; however, they are still expensive for some countries. In addition, only a few pathogens are commonly detected in the tests, and some viruses included in the large syndromic panels are rarely detected.[10] Therefore, it would also be important to include only the commonly isolated respiratory viruses in the molecular assay and to provide substantially less expensive molecular assays rather than detecting excessively various pathogens using large syndromic panels. In this point of view, the new EuDx ufPCR Flu & RSV would be beneficial for the rapid and cost-effective (about $20 per test) detection of common respiratory viruses.

In this study, we evaluated EuDx ufPCR Flu & RSV for detecting influenza A/B and RSV. The sensitivities of this assay were slightly lower than those of other RT-PCR assays; however, the agreements among the three respiratory virus assays were almost perfect for influenza A/B and RSV. In addition, the detection sensitivity of EuDx ufPCR Flu & RSV was excellent, and no cross-reactivity with other pathogens was observed. Therefore, we could conclude that the kit has the advantage of having an almost equivalent performance to available commercial molecular assays, a shorter turn-around time, cost-effectiveness, and portability.

In conclusion, we evaluated the recently developed respiratory PCR assays for the rapid detection of the most common respiratory viruses. Our results showed that EuDx ufPCR Flu & RSV had excellent performance for diagnosing influenza A/B and RSV infections. Therefore, we expect that this assay would offer clinical laboratories a valuable option for the rapid diagnosis and improved clinical management of potentially fatal respiratory viruses at a less expensive cost.

Declaration of Competing Interest

The authors declare that there is no conflict of interest with respect to the publication of this article.

Acknowledgments

We thank EUDIPIA for providing EuDx™ ufPCR Flu & RSV detection kits and EuDx™ GENECHECKER® ultra-fast PCR system. EUDIPIA only provided technical support and had no role in the study design, data collection, and interpretation.

References

1. Monto AS. Epidemiology of viral respiratory infections. *Am J Med* 2002;**112**(Suppl 6A):4S—12S.
2. Forum of International Respiratory Societies. *The global impact of respiratory disease*. 2nd ed. Sheffield: European Respiratory Society; 2017.
3. Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* 2017;**390**(10098):946—58.
4. Drysdale SB, Green CA, Sande CJ. Best practice in the prevention and management of paediatric respiratory syncytial virus infection. *Ther Adv Infect Dis* 2016;**3**(2):63—71.
5. WHO. *WHO recommendations on the use of rapid testing for influenza diagnosis*. 2005. https://www.who.int/influenza/resources/documents/rapid_testing/en/. [Accessed 1 August 2019].
6. Perk Y, Özdil M. Respiratory syncytial virüs infections in neonates and infants. *Türk Pediatri Arşivi* 2018;**53**(2):63.
7. *CLSI. User Protocol for evaluation of qualitative test performance, 2nd ed. CLSI guideline EP12-A2*. Wayne, PA: CLSI; 2008.
8. Popow-Kraupp T, Aberle JH. Diagnosis of respiratory syncytial virus infection. *Open Microbiol J* 2011;**5**:128—34.
9. Rheem I, Park J, Kim TH, Kim JW. Evaluation of a multiplex real-time PCR assay for the detection of respiratory viruses in clinical specimens. *Ann Lab Med* 2012;**32**(6):399—406.
10. Ye S, Wang T. Laboratory epidemiology of respiratory viruses in a large children's hospital: a STROBE-compliant article. *Medicine (Baltim)* 2018;**97**(30):e11385.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2020.06.012.