AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

Evaluation of a Real-Time Reverse Transcription-PCR (RT-PCR) Assay for Detection of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Clinical Samples from an Outbreak in South Korea in 2015

Jee-Soo Lee,[a,b] Ji Soo Ahn,[a] Byeong Su Yu,[a] Sung Im Cho,[a] Man Jin Kim,[a] Jong Moon Choi,[a,c] Soo Hyun Seo,[d] Sung Sup Park,[a] Moon-Woo Seong[a]

Department of Laboratory Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea[a]; Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Anyang, Republic of Korea[b]; Green Cross Genome, Green Cross Laboratories, Yong-in, Republic of Korea[c]; Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea[d]

In the period from September 2012 to April 2017, 1,936 laboratory-confirmed Middle East respiratory syndrome coronavirus (MERS-CoV) cases, including 690 deaths, were reported from 27 countries (http://www.who.int/emergencies/mers-cov/en/). Owing to the high virulence of MERS-CoV, there is an urgent need to develop rapid and reliable diagnostic assays for MERS-CoV detection (1, 2). Consequently, the WHO has recommended molecular detection of MERS-CoV, based on real-time reverse transcription-PCR (rRT-PCR) targeting of the genomic regions upstream of the E protein gene (upE) and open reading frame 1a (ORF1a), ORF1b, or the nucleocapsid (N) gene (3).

We evaluated the performance of a commercial MERS-CoV r gene rRT-PCR assay (Argene, bioMérieux, France) which targets the spike (S) glycoprotein gene. The S protein binds to the host cell receptor DPP4 and is essential for entry of the virus into the cell (4, 5). Herein, we report a comparison of the commercial assay with an in-house rRT-PCR assay, which targets upE and ORF1a per the WHO recommendation, using multiple clinical specimen types. A total of 130 respiratory samples that were previously analyzed using the in-house assay from June 2015 to September 2015 were examined. Of these, 50 samples (25 sputa, 9 oropharyngeal swabs [OPSs], 10 transtracheal aspirates [TTAs], 5 nasopharyngeal swabs [NPSs], and 1 endotracheal aspirate [ETA]) had been confirmed as MERS-CoV positive, and 80 samples (30 sputa, 19 OPSs, 20 TTAs, 10 NPSs, and 1 ETA) had been confirmed as MERS-CoV negative. RNA extraction was performed with an r gene RNA internal control using the MagNA Pure 96 RNA extraction kit (Roche, Switzerland), and 50-μl elution volumes were collected. Amplification was performed using the CFX96 real-time system (Bio-Rad, Hercules, CA, USA) in a reaction volume of 25 μl comprising 10 μl r gene premix, 5 μl r gene oligonucleotide primers and probes, and 10 μl RNA input. For the discrepant results, the in-house assay was additionally performed for confirmation. The in-house assay has been described previously (6, 7).

Of the 50 MERS-CoV-positive samples, 49 were deemed positive for MERS-CoV with the r gene rRT-PCR assay. The median threshold cycle (C_T) value for the S gene in the 49 positive samples was 34.3 (range, 20.23 to 42.49). C_T values for each specimen type are shown in Table 1. A strong correlation was observed between the S and upE C_T values in the positive samples (data not shown). The positive percent agreement,

Accepted manuscript posted online 31 May 2017

Citation Lee J-S, Ahn JS, Yu BS, Cho SI, Kim MJ, Choi JM, Seo SH, Park SS, Seong M-W. 2017. Evaluation of a real-time reverse transcription-PCR (RT-PCR) assay for detection of Middle East respiratory syndrome coronavirus (MERS-CoV) in clinical samples from an outbreak in South Korea in 2015. J Clin Microbiol 55:2554–2555. https://doi.org/10.1128/JCM.00667-17.

Editor Alexander J. McAdam, Boston Children's Hospital

Address correspondence to Moon-Woo Seong, mwseong@snu.ac.kr.

TABLE 1 Performance characteristics of the MERS-CoV r-gene rRT-PCR assay in comparison to those of the in-house assay[a]

MERS-CoV r-gene rRT-PCR assay specimen	No. of positive results/total no. of tests (%)		PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
	MERS-CoV-positive cases	MERS-CoV-negative cases			
Upper respiratory tract					
OPSs (n = 28)[b]	9/9 (100)	0/19 (0)	100.0 (70.1–100.0)	100.0 (83.2–100.0)	100.0 (87.9–100.0)
NPSs (n = 15)[c]	5/5 (100)	0/10 (0)	100.0 (56.6–100.0)	100.0 (72.2–100.0)	100.0 (79.6–100.0)
Lower respiratory tract					
Sputa (n = 55)[d]	24/25 (96.0)	0/30 (0)	96.0 (80.5–99.3)	100.0 (88.6–100.0)	98.2 (90.4–99.7)
TTAs (n = 30)[e]	10/10 (100)	0/20 (0)	100.0 (72.2–100.0)	100.0 (83.9–100.0)	100.0 (88.6–100.0)
ETAs (n = 2)[f]	1/1 (100)	0/1 (0)	100.0 (20.7–100.0)	100.0 (20.7–100.0)	100.0 (34.2–100.0)

[a]MERS-CoV, Middle East respiratory syndrome coronavirus; rRT-PCR, real-time reverse transcription-PCR; PPA, positive percent agreement; NPA, negative percent agreement; OPA, overall percent agreement; CI, confidence interval; OPSs, oropharyngeal swabs; NPSs, nasopharyngeal swabs; TTAs, transtracheal aspirates; ETAs, endotracheal aspirates.
[b]The median C_T (range) was 39.2 (30.4 to 42.5).
[c]The median C_T (range) was 29.9 (27.6 to 38.1).
[d]The median C_T (range) was 33.5 (20.2 to 41.0).
[e]The median C_T (range) was 32.5 (22.3 to 37.5).
[f]The median C_T was 35.8.

negative percent agreement, and overall percent agreement of the r gene rRT-PCR assay were 98.0%, 100%, and 99.2%, respectively, when the in-house assay was used as the gold standard. Results for each specimen type are summarized in Table 1. According to our previous study (6), the S gene mutation was not observed in the sputum sample showing discordant results. Consequently, the confirmatory in-house assay was performed, which revealed that the discordant sample had a very low viral load (upE C_T, 40.7; ORF1a C_T, 41.0).

Our data demonstrate that the r gene rRT-PCR assay is comparable to the WHO-recommended in-house rRT-PCR assay for a large number of patient specimens. The good performance of this commercial diagnostic assay warrants its application in clinical settings, especially considering that commercial assays may provide broader access and be more cost-effective for routine diagnosis. In conclusion, the r gene rRT-PCR assay is a reliable method to detect MERS-CoV and is a suitable alternative to the in-house rRT-PCR assay.

ACKNOWLEDGMENTS

This evaluation was supported by a research grant from bioMérieux, France. bioMérieux had no role in data collection or interpretation.

REFERENCES

1. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 367:1814–1820. https://doi.org/10.1056/NEJMoa1211721.
2. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, Flemban H, Al-Nassir WN, Balkhy HH, Al-Hakeem RF, Makhdoom HQ, Zumla AI, Memish ZA. 2013. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. Lancet Infect Dis 13:752–761. https://doi.org/10.1016/S1473-3099(13)70204-4.
3. Al Johani S, Hajeer AH. 2016. MERS-CoV diagnosis: an update. J Infect Public Health 9:216–219. https://doi.org/10.1016/j.jiph.2016.04.005.
4. Wang N, Shi X, Jiang L, Zhang S, Wang D, Tong P, Guo D, Fu L, Cui Y, Liu X, Arledge KC, Chen YH, Zhang L, Wang X. 2013. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Res 23:986–993. https://doi.org/10.1038/cr.2013.92.
5. Wang Q, Wong G, Lu G, Yan J, Gao GF. 2016. MERS-CoV spike protein: targets for vaccines and therapeutics. Antiviral Res 133:165–177. https://doi.org/10.1016/j.antiviral.2016.07.015.
6. Seong MW, Kim SY, Corman VM, Kim TS, Cho SI, Kim MJ, Lee SJ, Lee JS, Seo SH, Ahn JS, Yu BS, Park N, Oh MD, Park WB, Lee JY, Kim G, Joh JS, Jeong I, Kim EC, Drosten C, Park SS. 2016. Microevolution of outbreak-associated Middle East respiratory syndrome coronavirus, South Korea, 2015. Emerg Infect Dis 22:327–330. https://doi.org/10.3201/eid2202.151700.
7. Kim SY, Park SJ, Cho SY, Cha RH, Jee HG, Kim G, Shin HS, Kim Y, Jung YM, Yang JS, Kim SS, Cho SI, Kim MJ, Lee JS, Lee SJ, Seo SH, Park SS, Seong MW. 2016. Viral RNA in blood as indicator of severe outcome in Middle East respiratory syndrome coronavirus infection. Emerg Infect Dis 22:1813–1816. https://doi.org/10.3201/eid2210.160218.