



# 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

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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- $\mu$ l elution volumes were collected. Amplification was performed using the CFX96 real-time system (Bio-Rad, Hercules, CA, USA) in a reaction volume of 25  $\mu$ l comprising 10  $\mu$ l r gene premix, 5  $\mu$ l r gene oligonucleotide primers and probes, and 10  $\mu$ l 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,

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**TABLE 1** Performance characteristics of the MERS-CoV r-gene rRT-PCR assay in comparison to those of the in-house assay<sup>a</sup>

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) <sup>b</sup>	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) <sup>c</sup>	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) <sup>d</sup>	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) <sup>e</sup>	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) <sup>f</sup>	1/1 (100)	0/1 (0)	100.0 (20.7–100.0)	100.0 (20.7–100.0)	100.0 (34.2–100.0)

<sup>a</sup>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.

<sup>b</sup>The median  $C_T$  (range) was 39.2 (30.4 to 42.5).

<sup>c</sup>The median  $C_T$  (range) was 29.9 (27.6 to 38.1).

<sup>d</sup>The median  $C_T$  (range) was 33.5 (20.2 to 41.0).

<sup>e</sup>The median  $C_T$  (range) was 32.5 (22.3 to 37.5).

<sup>f</sup>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.

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