Diagnostic performmace evaluation of four commercial Middle East Respiratory Syndrome Corona Virus (MERS-CoV) real-time RT-PCR assays

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Background:

Since the idenfication of Middle East Respiratory Syndrome corona virus (MERS-CoV), a novel human beta-coronavirus causing severe acute respiratory infection [1], several molecular amplification assays for MERS-CoV surveillance have been developed [2-6]. However, validation of these assays have been limited to either virus spiked mock samples or small numbers of confirmed MERS-CoV clinical specimens. Our data presents the largest panel of confirmed positive clinical specimens used to evaluate the clinical sensitivity of MERS-CoV real-time RT-PCR (rRT-PCR) assays to date. We describe the performance evaluation of four commercial MERS-CoV rRT-PCR assays on clinical specimens collected during the period surrounding the 2015 outbreak in Riyadh, Saudi Arabia.

Material/methods:

Samples: 34 archived nasopharyngeal swabs in viral transport medium from 18 confirmed MERS-CoV positive cases, and a further 200 randomly selected MERS-CoV negative clinical specimens from routine surveillance of patients presenting with probable/suspected diagnosis of MERS-CoV, attending King Kahlid University Hospital, Riyadh between 1 January and 30 April 2015. An additional 22 diverse clinical respiratory specimens, were also included to evaluate assay cross reactivity.

Nucleic Acid extraction: MagNA Pure Compact system, using the Nucleic Acid Isolation Kit I (Roche Applied Science) under default instrument settings. Extractions were performed on 200µL of each specimen, with a final elution volume of 60µl.

Table 1 Commercial MERS-CoV rRT-PCR assays

Characteristic	Assay 1	Assay 2	Assay 3	Assay 4
Manufacturer	AltonaDiagnosticsRealStar® MERS-CoV RT-PCR Kit	TIBMolBiolModularDx Kit Coronavirus SA1 (EMC) upstream E-gene **and** TIBMolBiolModularDx Kit MERS-Coronavirus (EMC) Orf1a	PrimerDesign™Genesig® Kit for Human Coronavirus 2012 (HCoV_2012)	Seegene MERS-CoV detection
Master Mix supplier (if different)	-	*Roche Realtime Ready RNA Virus Master*	-	-
Assay signature	UpE Orf1a	UpE Orf1a	ORF5/E ORF1ab	Not disclosed
Internal control	Yes	No	Yes	Yes
Nucleic acid input volume	10 µl	5 µl	8 µl	5µl
Total reaction volume	25 µl	20 µl	20 µl	25 µl
Limit of detection	Orf1a: 0.93 copies/µl UpE: 0.54copies/µl	10 copies/reaction	<100 copies/reaction	Not disclosed
Thermal cycler	Qiagen Rotor-Gene Q® 3000	Roche LightCycler® 2.0 Instrument	Roche Lightcycler® 480	BioRad CFX
PCR time	2.5 hr	45-50 mins	2.5 hr	2.5 hr
Analysis type	Semi-quantitative	Semi-quantitative	Quantitative	Qualitative

Table 2 MERS-CoV rRT-PCR assay performance summary

Assay	% Sensitivity	% Specificity	% Positive Predictive Value (95% CI)	% Negative Predictive Value (95% CI)
1	100 (34/34)	100 (222/222)	100 (89.72-100)	100 (98.35-100)
2	94.12 (32/34)	99.55 (221/222)	96.97 (84.24-99.92)	99.10 (96.80-99.89)
3	41.18 (14/34)	100 (222/222)	100 (76.84-100)	91.74 (87.52-94.88)
4	79.41 (27/34)	100 (222/222)	100 (87.23-100)	96.94 (93.80-98.76)

Results:

The Altona diagnostic assay correctly identified 100% (34/34) of confirmed positive specimens, with 100% specificity. The TIBMolBIOL/Roche assay displayed 94% sensitivity and 99% specificity in our clinical specimen pool. The specificity of both Seegene and GeneSig assays was 100%. However, the GeneSig assay lacked sensitivity with our clinical specimens (41%), particularly with specimens displaying low viral loads. Comparatively, Seegene displayed a slightly better sensitivity of 79%

Conclusions:

This report demonstrates the diagnostic sensitivity to four commercial MERS-CoV rRT-PCR assays on archived clinical specimens. Discordant results between the assays were seen with those specimen containing low MERS-CoV concentrations. Nevertheless, the Altona assay proved to be the most sensitive. Although ultimately MERS-CoV screening cannot rely on a single assay, studies such as these may contribute towards improving diagnostic assay performance.

References:

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