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Clinical validation of 3 commercial real-time reverse transcriptase polymerase chain reaction assays for the detection of Middle East respiratory syndrome coronavirus from upper respiratory tract specimens

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ABSTRACT

Since discovery of Middle East respiratory syndrome coronavirus (MERS-CoV), a novel betacoronavirus first isolated and characterized in 2012, MERS-CoV real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays represent one of the most rapidly expanding commercial tests. However, in the absence of extensive evaluations of these assays on positive clinical material of different sources, evaluating their diagnostic effectiveness remains challenging. We describe the diagnostic performance evaluation of 3 common commercial MERS-CoV rRT-PCR assays on a large panel (n = 234) of upper respiratory tract specimens collected during an outbreak episode in Saudi Arabia. Assays were compared to the RealStar® MERS-CoV RT-PCR (Alton Diagnostics, Hamburg, Germany) assay as the gold standard. Results showed i) the TIB MolBiol® LightMix *UpE* and *Orf1a* assays (TIB MolBiol, Berlin, Germany) to be the most sensitive, followed by ii) the Anyplex™ Seegene MERS-CoV assay (Seegene, Seoul, Korea), and finally iii) the PrimerDesign™ Genesig® HCoV_2012 assay (PrimerDesign, England, United Kingdom). We also evaluate a modified protocol for the PrimerDesign™ Genesig® HCoV_2012 assay.
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1. Introduction

Middle East respiratory syndrome corona virus (MERS-CoV), a novel human betacoronavirus, was first isolated in 2012 following the fatality of a patient with severe acute respiratory infection in Saudi Arabia (Zaki et al., 2012). The genomic sequencing and public release of that isolate (van Boheemen et al., 2012) enabled the development of several real-time reverse transcriptase polymerase chain reaction (rRT-PCR)–based assays to facilitate laboratory detection and confirmation of MERS-CoV RNA in clinical specimens. Screening assays have primarily targeted genomic regions upstream of the envelope gene (*UpE*), while confirmatory assays have targeted viral open reading frames 1a (*ORF1a*), *ORF1b*, or both (*ORF1ab*) (Corman et al., 2012a, b). More recently, alternative genomic targets have included the nucleocapsid (N) (Lu et al., 2014) and RNA-dependent RNA polymerase genes (Corman et al., 2012b). Following deployment of these molecular assays, reports of MERS-CoV cases continue to rise both regionally and globally. According to WHO statistics, as of 31 August 2016, a total of 1800 laboratory confirmed MERS-CoV cases including 640 related deaths have been reported in 27 countries. Rapid and reliable detection of MERS-CoV is therefore essential to controlling the spread of this emerging threat.

In the event of a MERS-CoV outbreak, the World Health Organization (WHO) have authorized emergency use of 2 rRT-PCR assays for MERS-CoV detection, i) the RealStar® MERS-CoV RT-PCR assay (Corman et al., 2014), a commercially available assay produced by Altona Diagnostics (Hamburg, Germany) and ii) the CDC Novel Coronavirus 2012 Real-time RT-PCR assay (Lu et al., 2014), distributed free of charge to qualified laboratories by the Centers of Disease Control and Prevention (Atlanta, GA, USA). During the initial stages of MERS-CoV spread, these assays enabled rapid diagnosis to support the public health response to MERS-CoV. However, as with other currently available commercial assays, evaluation of the clinical sensitivities of these kits has been limited to virus spiked mock samples or small numbers of clinical specimens from MERS-CoV infected cases. Due to the recent increase in commercially available MERS-CoV rRT-PCR kits (n = 16) (Pas et al., 2015), there is a need to evaluate assay performances on different specimen types to better understand their diagnostic effectiveness.

Lower respiratory tract (LRT) specimens are known to contain high viral loads. However, upper respiratory tract (URT) specimens such as

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nasopharyngeal/oropharyngeal (NPOP) swabs remain the easiest and most common specimen type collected from patients experiencing respiratory distress. This article describes the performance evaluation of 3 commonly used commercial MERS-CoV rRT-PCR assays and 1 modified protocol, on a large panel of URT specimens collected during the period surrounding an outbreak episode in Riyadh, Saudi Arabia.

2. Materials and methods

2.1. Specimens

During the late winter to early spring period of 2015, King Khalid University Hospital (KKUH), Riyadh (Saudi Arabia) observed an outbreak of MERS-CoV infections. Specimens were screened by RT-PCR using the RealStar® MERS-CoV rRT-PCR assay. Laboratory-confirmed samples were also subjected to deep sequencing and phylogenetic analysis as previously described (Somily et al.).

For this study, archived combined NPOP swabs in viral transport medium (VTM; Copan, Brescia, Italy) from patients presenting with probable or suspected diagnosis of MERS-CoV, attending KKUH in the period surrounding the outbreak (1 January and 30 April 2015) were obtained from the Molecular Diagnostic Unit, KKUH. This included 34 archived NPOP swabs (in VTM) from 18 sequence-confirmed MERS-CoV–positive cases, and a further 200 randomly selected MERS-CoV–negative NPOP swabs. Aliquots of VTM from the original frozen NPOP tubes of these specimens were prepared in 2.0-mL tubes. All specimens were delinked from patient details, reissued unique study identifiers, reextracted, and concurrently subjected to MERS-CoV rRT-PCR as described below. The researcher performing testing was blinded to the original MERS-CoV screening outcomes of these 234 specimens. The RealStar® MERS-CoV rRT-PCR assay was used as the gold standard. The results of the RealStar® MERS-CoV assay in Tables 2–3 represent the results from the reextracted specimens, for comparative analysis.

An additional 22 diverse clinical and proficiency testing specimens, previously positive for other respiratory viruses using the Anyplex™II RV16 detection assay (Seegene, Seoul, Korea) (Section 2.4.1), were also included in this study to evaluate assay cross-reactivity. This study was approved by, and performed according to the guidelines of The KKUH and College of Medicine institutional review board committee under project number E16–1886.

2.2. RNA extraction

Total nucleic acid extractions from VTM aliquots were performed on the MagNA Pure Compact system (Roche Applied Science, Bavaria, Germany), using the Nucleic Acid Isolation Kit I and the default instrument settings. Extractions were performed on 300 μL of each specimen, with a final elution volume of 50 μL. Duplicate extractions were performed for each specimen and the final elutions are pooled into one 100-μL extract for concurrent use.

2.3. MERS-CoV rRT-PCR assays

Table 1 summarizes the specifications of all the MERS-CoV rRT-PCR assays used in this study. Amplifications were performed according to the manufacturer's instructions. All assays were supplied as complete kits, inclusive of master mix reagents and internal control, with the exception of the TIB MolBiol® LightMix kits (TIB MolBiol, Berlin, Germany), which can be used with the LightCycler® Multiplex RNA Virus Master Mix (Roche Applied Science) (Assay 2). Assay signatures (except Anyplex™ Seegene, discussed below) targeted the *UpE* genomic region for MERS-CoV screening, and the *Orf1a* or *Orf1ab* genes for confirmatory investigations. Positive *UpE* and *Orf1a* amplification confirmed MERS-CoV infection. For the purposes of this study, all specimens were analyzed on both the screening and confirmatory assays. Additionally, internal controls were spiked in the PCR mixtures according to each manufacturer's instructions.

A modification of the PrimerDesign™ Genesig® assay was also evaluated. The unmodified assay (assay 3A) reaction composition was as follows: 10 μL of Oasig™ One-Step 2× qRT-PCR Master Mix (Primer Design) was added to 1 μL of HCoV_2012 primer/probe mix, 1 μL of internal extraction control primer/probe mix, and 8 μL of the extracted sample RNA. For the controls, only 5-μL control material was used, and the reaction volume adjusted with water. Amplification was performed on the LightCycler® 480 system using the thermocycling conditions specified by GeneSig®. For the modified protocol (assay 3B), the LightCycler® Multiplex RNA Virus Master Mix was used in the place of

Table 1
MERS-CoV rRT-PCR assay specifications.

Characteristic	Assay 1	Assay 2	Assay 3A	Assay 3B (modified 3A)	Assay 4
Manufacturer/ Assay details	RealStar® MERS-CoV RT-PCR assay	TIB MolBiol® LightMix ModularDx Kit Coronavirus SA1 (Erasmus Medical Center [EMC]) upstream E-gene assay and TIB MolBiol® ModularDx Kit MERS-Coronavirus (EMC) *Orf1a* assay	PrimerDesign™ Genesig® Kit for Human Coronavirus 2012 (HCoV_2012) assay (PrimerDesign, England, United Kingdom)	PrimerDesign™ Genesig® Kit for Human Coronavirus 2012 (HCoV_2012) assay	Anyplex™ Seegene MERS-CoV detection assay
Master Mix supplier (if different)		Roche Realtime Ready RNA Virus Master (Roche Applied Science)		Roche Realtime Ready RNA Virus Master	
Assay signature	*UpE* *Orf1a*	*UpE* *Orf1a*	*ORF5/E* *ORF1ab*	*ORF5/E* *ORF1ab*	Not disclosed
Internal control	Yes	No	Yes	Yes	Yes
Nucleic acid input volume[a]	10 μL	5 μL	8 μL	5 μL	5 μL
Total reaction volume[a]	25 μL	20 μL	20 μL	20 μL	25 μL
Limit of detection[b]	*Orf1a*: 0.93 copies/μL *UpE*: 0.54copies/μL	10 copies/reaction	<100 copies/reaction	Not determined	Not disclosed
Thermal cycler	Qiagen Rotor-Gene Q® 3000 (Germantown, MD, USA)	Roche LightCycler® 2.0 Instrument (Roche Applied Science)	Roche LightCycler® 480 (Roche Applied Science)	Roche LightCycler® 480	BioRad CFX (Hercules, CA, USA)
PCR time	2.5 h	45–50 min	2.5 h	2.5 h	2.5 h
Analysis type	Semi-quantitative	Semi-quantitative	Quantitative	Semi-Quantitative	Qualitative

[a] *UpE* and *Orf1a* reactions were performed separately.
[b] Limit of detection values is manufacturer-specified values.

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