P0100
Paper Poster Session
Emerging and pre-emerging viruses

Clinical evaluation of commercial Middle East Respiratory Syndrome corona virus 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, several molecular amplification assays for MERS-CoV surveillance have been developed. 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:

Thirty four archived clinical specimens (nasopharyngeal swabs in viral transport medium) from 18 confirmed MERS-CoV positive cases, and a further 200 randomly selected MERS-CoV negative (by PCR) clinical specimens from routine surveillance of patients presenting with probable or suspected diagnosis of MERS-CoV, attending King Kahlid University Hospital, Riyadh between 1 January and 30 April 2015 were included in this study. An additional 22 diverse clinical respiratory specimens, were also included to evaluate assay cross reactivity. Total nucleic acid extractions were performed on the MagNA Pure Compact system, using the Nucleic Acid Isolation Kit I (Roche Applied Science) and the default instrument settings. Extractions were performed on 200µL of each specimen, with a final elution volume of 60µl.

Four commercial one-step rRT-PCR assays were evaluated for the detection of MERS-CoV RNA: (i) Altona Diagnostics RealStar® MERS-CoV RT-PCR Kit, (ii) TIBMolBiol ModularDx Kit Coronavirus SA1 (EMC) upstream E-gene and Orf1a kits, (iii) PrimerDesign™Genesig® Kit for Human Coronavirus 2012 (HCoV_2012), and (iv) Seegene MERS-CoV detection kit. Amplifications were performed according to the manufacturer's instructions.

Results:

The Altona diagnostic assay correctly identified 100% (34/34) of confirmed positive specimens, with 100% specificity. The TIBMolBIOL/Roche assay displayed 94.12% sensitivity and 99.54% 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 (41.2%), particularly with

specimens displaying low viral loads. Comparatively, Seegene displayed a slightly better sensitivity of 79.4%

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 (high C_T values). 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.