Use of the Human Coronavirus 2012 (MERS) GeneSig kit for MERS-CoV detection

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ABSTRACT

Introduction: Mortality due to MERS-CoV infection is common especially among immunocompromised patients. The pathogenesis and the transmission mode of this virus are still not well understood. The name of the virus is derived from the area of its appearance and the genomic sequence that was used in the development of qRT-PCR assays for MERS-CoV detection was retrieved from the first detected case isolate. The employed assays target various regions including the area upstream of the envelope gene (upE) that is used for screening and the open reading frames (ORF) 1a and 1b used for confirmation.

Aim: This study assesses the use of a MERS-CoV specific assay for screening of respiratory samples in anticipation of the possible spread of the virus in the region.

Methods: 46 respiratory specimens were tested using the qualitative one-step qRT-PCR GeneSig Human Coronavirus 2012 (MERS) kit (PrimerDesign™).

Results: Out of the 46 tested samples, 45 were negative for MERS-CoV and one sample was found MERS-CoV positive.

Conclusion: The GeneSig Human Coronavirus 2012 (MERS) kit is very useful for the screening of suspected respiratory cases in the Middle East area as well as other regions.

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1. Introduction

The Middle East Respiratory Syndrome (MERS) is a novel Coronavirus first discovered in September 2012 in a cell-culture from a patient after his death from pneumonia in June 2012 in Saudi Arabia (Assiri et al., 2013). Mortality due to MERS-CoV infection is common especially among immunocompromised patients (Al-Abdallat et al., 2014).

Coronaviruses are the largest positive single-stranded RNA viruses with a genome that can reach up to 32 kb long. The genome entails a 5′-terminal cap structure and is polyadenylated at its 3′ end, and is occupied by two large overlapping open reading frames, ORFs 1a and 1b (Subissi et al., 2014). MERS-CoV is part of the C lineage of the Betacoronavirus genus and it is related to the HKU4 and HKUS coronaviruses detected in bats (Omarani et al., 2013).

MERS-CoV and highly homologous MERS-like CoV were isolated in dromedary camels and African Neoromicia capensis bats, respectively. Although MERS-CoV is known to have zoonotic origins the pathway of transmission from the animal reservoir to humans is still not well understood. It is, however, suggested that a bat to camel to human route could explain the MERS-CoV 2012 outbreak in humans, due to the emerging camel trade between equatorial Africa and Saudi Arabia during the last 20 years (Gralinski and Baric, 2015).

MERS-CoV causes severe lower respiratory tract infection that sometimes requires admission to the intensive care unit. Patients usually respond well to alpha interferon or cyclosporine treatment, however, no specific antiviral or vaccine is available for MERS-CoV (Ohnuma et al., 2013). More than 1540 cases from 21 countries worldwide have been infected and diagnosed with MERS-CoV since June 2012, and almost a third of those died (Saad et al., 2014; WHO, c 2015).

Patients with MERS-CoV infection tend to have leukocytosis, lymphopenia and some express thrombocytopenia and coagulopathy. Moreover, creatinine levels, lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase levels may be elevated and thus implying a liver or kidney disease (van den Brand et al., 2015).

Males and elderly are more commonly infected with MERS-CoV than females and younger people, and human-to-human transmission in the community is mainly in healthcare settings where poor infection control measures are implemented especially in intensive care units, and where overcrowding of patients may occur (Saad et al., 2014). Other reported cases of MERS-CoV, especially the ones reported in Europe or the US are more likely associated with the travel history to the Middle East. The rate transmission of the virus from infected patients to their household contacts is as low as 5% (Drosten et al., 2014).
Rapid detection and implementation of proper infection control measures are two essential steps to limit and control MERS-CoV transmission in healthcare settings. This includes applying contact and airborne precautions such as wearing gloves, gowns and surgical masks upon entry to a room of MERS-CoV infected patient in isolation and removing such personal protective equipment upon leaving the room (Zumla and Hui, 2014). Protective measures outside healthcare settings include avoiding contact with dromedary camels, the host of MERS-CoV, and restraining from drinking camel urine or raw milk or eating raw or undercooked camel meat. Moreover people working with camels must wear protective clothing and facial masks, and maintain a good personal hygiene (Reusken et al., 2014).

Ideally, lower respiratory specimens such as sputum, bronchoalveolar lavage, bronchial wash and tracheal aspirates are the most representative and appropriate for MERS-CoV testing (CDC, June 14, 2013).

The name of the virus is derived from the area of its appearance and the genomic sequence that was used in the development of qRT-PCR assays for MERS-CoV detection was retrieved from the first detected case isolate (Lu et al., 2014). The employed assays target various regions including the area upstream of the envelope gene (upE) that is used for screening and the open reading frames (ORF) 1a and 1b used for confirmation (Memish et al., 2014).

The aim of this study was to assess the use of a MERS-CoV specific assay for screening of respiratory samples referred to a major tertiary care center in anticipation of the possible spread of the virus in the region.

2. Materials and methods

2.1. Samples

RNA was extracted from 46 respiratory specimens using the QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) that does not use phenol/chloroform extraction or alcohol precipitation protocols. Briefly, a lysis buffer was used to isolate non-degraded viral RNA and inactivate...
RNAs. The sample was then transferred to the QIAamp Mini spin column under optimal conditions that ensured effective binding of the RNA to the QIAamp membrane. Possible contaminants were removed using two different wash buffers, then, a RNase-free buffer was employed to elute the RNA.

2.2. Real-time PCR

MERS-Coronavirus qualitative one-step qRT-PCR testing was done using the GeneSig Human Coronavirus 2012 (MERS) kit (PrimerDesign™, London, UK). The HCoV primers are designed for the specific and exclusive in vitro detection of the novel coronavirus isolates based on sequences for the EMC strain by targeting the ORF1ab and the upE regions of ORF5. The ORF1ab and ORF5/E primer and probe sets are designed to be specific to the MERS strain regardless of the species of origin and are detected through the VIC channel.

An internal RNA extraction control is available in the kit and was added to the lysis buffer as an external source of RNA template, during the extraction step, and co-purified with the sample RNA to monitor this process as well as to rule out the presence of inhibitors in the real-time PCR step. For the latter purpose, a separate mix of primers for the internal control, with limited concentrations that allow for multiplexing with the target sequence primers without affecting the ability to detect the target even when present in low concentrations, was used. The detection of the internal control was through the VIC channel.

Moreover, a primer mix for the endogenous control Actin Beta (ACTB) gene was used to evaluate the quality of the original biological sample. The detection of ACTB is through the VIC channel, thus a separate reaction mix than that of the target is necessary to test for ACTB as multiplexing for ACTB and HCoV primers is not possible.

For each sample, three reaction mixes were prepared. For the detection of ORF1ab and ORF5/E two 20 μl reaction tubes were prepared as follows: 10 μl of oasis™ qRT-PCR mastermix, 1 μl of HCoV-2012 (ORF1ab or ORF5/E) primer/probe mix, 1 μl of internal extraction control primer/probe mix, 6 μl of RNA/DNase free water and 2 μl RNA material. To detect the endogenous ACTB control, a separate 20 μl reaction mix was prepared, containing 10 μl of oasis™ qRT-PCR mastermix, 1 μl of endogenous ACTB primer/probe mix, 7 μl of RNA/DNase free water and 2 μl RNA material. The amplification conditions involved a reverse transcription step at 42 °C for 10 min, an enzyme activation step at 95 °C for 2 min and 50 cycles of 95 °C for 10 s followed by 60 °C for 60 s. A positive control for ORF1ab and ORF5/E, as well as a negative control (RNase/DNase free water) was used with each run.

2.3. Interpretation of results

The test requires 65 min hands-on time and the PCR protocol lasts two hours after which the ROTORGENE-Q (Qiagen, UK) Software is used for analysis.

3. Results

Out of the 46 tested samples, 45 were negative for MERS-CoV and one sample was found MERS-CoV positive. The positive result was validated by the Naval Medical Research Unit (NAMRU) laboratory, located in Cairo-Egypt, in collaboration with the World Health Organization (WHO). All other results were validated with a CAP accredited referral laboratory in Germany and the concordance rate was 100%.

Fig. 1 represents a graph of the amplification of the MERS-CoV RNA and the endogenous ACTB in the MERS-CoV positive sample. Positive and negative controls were used and signal detection was done on the FAM channel. The graph in Fig. 2 represents the detection of the internal RNA extraction control (IEC) in the fluorescence channel VIC, for the same positive sample. IEC was only added to the sample during extraction and not to the positive or negative controls. An acceptable Ct value of the IEC ranges between 28 and 34.

4. Conclusion

The GeneSig Human Coronavirus 2012 (MERS) kit is a rapid and useful kit for the screening of suspected respiratory cases in the Middle East area as well as other regions. The collaboration of different molecular microbiology laboratories with the World Health Organization (with viral cultures done as gold standard reference testing) has always been a rewarding and teaching experience. No extensive sensitivity and specificity work-up has been performed from our side on multiple samples due to the presence of a single MERS-CoV positive case; however, our lower limit of detection is compatible with the 100 copies/ml reported by the manufacturer. The clinicians have advised that this detection limit is acceptable for their management algorithms.

To our knowledge, this is the first report that describes the clinical application of the GeneSig kit in the MERS-CoV epidemic era.

References


