Verification and Validation of the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0

Hussein El Halas[1], Stephan Ölschläger[1], and Leonie-Sophie Hecht[1]
[1]altona Diagnostics GmbH, Hamburg, Germany

Introduction

The Middle East Respiratory Syndrome (MERS) is a respiratory disease caused by a corona virus called MERS-CoV. The virus was first isolated in 2012 in Saudi Arabia, the place where the majority of cases have been reported.

MERS-CoV is a zoonotic disease and it is most likely that (African) bats are the original host species. From bats the virus spread to camels which became a new reservoir for MERS-CoV. Many human cases have been directly or indirectly linked to contact with camels. It is likely that the virus has spread from camels to humans. However, the exact way of the virus transmission is still not well understood and yet to be determined (1).

The WHO recommends MERS-CoV RT-PCR positive samples to be confirmed with a second assay targeting other virus genome region.
In addition to the RealStar® MERS-CoV RT-PCR Kit 1.0 targeting a region upstream of the E gene (upE) and other targeting open reading frame 1a (orf1a), altona Diagnostics GmbH has developed the RealStar® Mers-CoV (N gene) RT-PCR Kit 1.0 targeting the N gene as a method to screen and confirm samples with discordant results (2, 3).
Here, we present data on the analytical sensitivity and specificity of the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0.

Materials and Methods

The RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0 limit of detection was determined using probit analysis after testing replicates of limited dilutions of quantified *in vitro* transcribed RNA containing the RT-PCR target sequence.
The analytical specificity of the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0 was evaluated by testing a panel of genomic RNA/DNA extracted from different pathogens that are related to MERS-CoV and/or can cause symptoms similar to MERS-CoV. Moreover, the assay specificity was also evaluated by testing the MERS-CoV QCMD 2016 and MERS-CoV QCMD 2017 panels.

Results

Analytical Sensitivity:

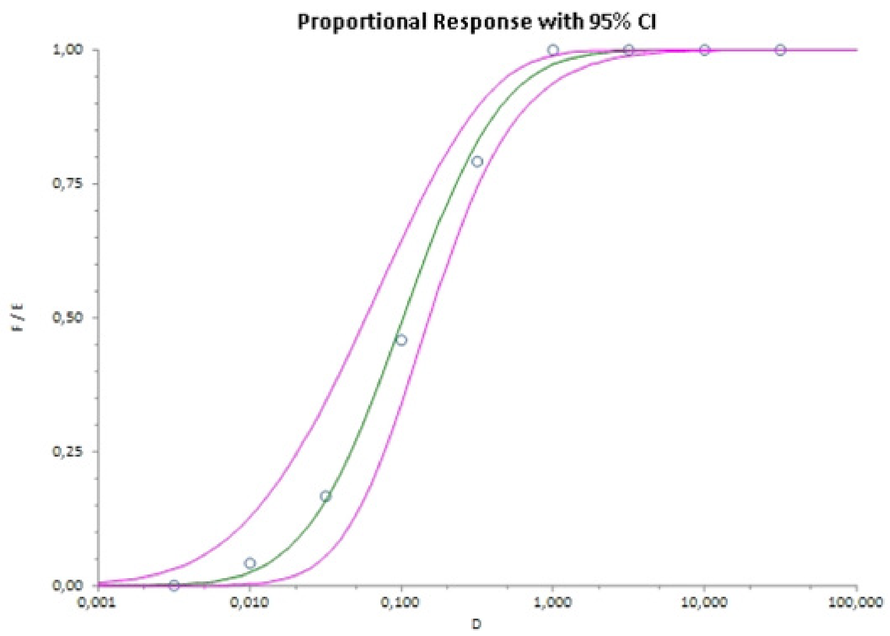

Figure 1: Probit analysis for the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0

The analytical sensitivity (95% Limit of Detection, LoD95) was determined by testing replicates of half-logarithmic dilutions of virus specific *in vitro* transcribed RNA. The X-axis shows the concentration of RNA and the Y-axis the proportion of positive results. The limit of detection for MERS-CoV using the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0 is **0.71 copies/µl** (95% confidence interval from 0.43 to 1.62 copies/µl).

Analytical Specificity:

Table 1: The RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0 did not cross-react with any of the following pathogens:

Pathogen	MERS-CoV (N gene)	Internal Control
Adenovirus	Negative	Valid
Bordetella pertussis	Negative	Valid
Chlamydophila pneumoniae	Negative	Valid
Haemophilus influenzae	Negative	Valid
hMPV	Negative	Valid
Human coronavirus NL63	Negative	Valid
Human coronavirus 229E	Negative	Valid
Human coronavirus OC43	Negative	Valid
Human coronavirus HKU1	Negative	Valid
Influenza A Virus	Negative	Valid
Influenza B virus	Negative	Valid
Klebsiella pneumoniae	Negative	Valid
Legionella pneumophila	Negative	Valid
Mycobacterium tuberculosis	Negative	Valid
Mycoplasma pneumoniae	Negative	Valid
Parainfluenza virus	Negative	Valid
Respiratory Syncytial Virus	Negative	Valid
Rhinovirus	Negative	Valid
SARS-CoV	Negative	Valid
Streptococcus pneumoniae	Negative	Valid
Streptococcus pyogenes	Negative	Valid

Table 2: MERS Coronavirus QCMD 2016 panel Results:

Sample Code	Sample Content	Sample Status	Detection Frequency	Expected Results	Results RealStar® Kit
MERS16- 01	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS16- 02	Coronavirus - OC43	CORE	Negative	–	–
MERS16- 03	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS16- 04	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS16- 05	Coronavirus Negative	CORE	Negative	–	–
MERS16- 06	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS16- 07	Coronavirus - NL63	CORE	Negative	–	–
MERS16- 08	MERS Coronavirus	CORE	Frequently Detected	+	+

QCMD 2016 samples panel were correctly identified by the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0.

Table 3: MERS Coronavirus QCMD 2017 panel Results:

Sample Code	Sample Content	Sample Status	Detection Frequency	Expected Results	Results RealStar® Kit
MERS17S- 01	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS17S- 02	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS17S- 03	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS17S- 04	MERS Coronavirus	CORE	Frequently Detected	+	+
MERS17S- 05	Coronavirus Negative	CORE	Negative	–	–
MERS17S- 06	Coronavirus - OC43	CORE	Negative	–	–
MERS17S- 07	Coronavirus - NL63	CORE	Negative	–	–
MERS17S- 08	MERS Coronavirus	CORE	Frequently Detected	+	+

QCMD 2017 samples panel were correctly identified by the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0.

Conclusion

The results presented here show that the RealStar® MERS-CoV (N gene) RT-PCR Kit 1.0 is a reliable assay, which can complement the RealStar® MERS-CoV RT-PCR Kit 1.0 for screening and confirmation of MERS-CoV infections.

References

1) Goldstein, Stephen A., and Susan R. Weiss. “Origins and Pathogenesis of Middle East Respiratory Syndrome-associated Coronavirus: Recent Advances.” F1000Research 6 (2017): 1628. doi:10.12688/f1000research.11827.1.
2) “WHO MERS-CoV Global Summary and Assessment of Risk” 21 July 2017.
3) Laboratory Testing for Middle East Respiratory Syndrome Coronavirus Interim guidance (revised) WHO, January 2018.

Contact

leonie.hecht@altona-diagnostics.com
altona Diagnostics GmbH, Mörkenstr. 12, 22767 Hamburg, Germany