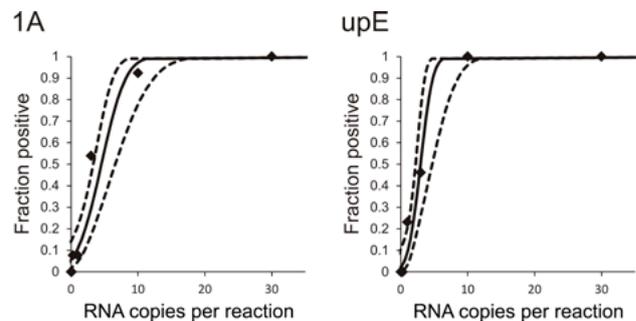# RealStar® MERS-CoV RT-PCR Kit 1.0

In 2012, a new pathogen emerged in the Middle East, causing severe respiratory infections with a high fatality rate among the patients. Middle East respiratory syndrome-coronavirus (MERS-CoV) is still mainly spreading in Saudi-Arabia and other countries of the Arabian Peninsula but has already reached many other countries worldwide (e.g. USA, Germany, Malaysia). Until April 2014, the number of infections remained relatively low but increased dramatically since then.

In collaboration with the group of Prof. Dr. Christian Drosten (Institute of Virology, University of Bonn Medical Center, Bonn, Germany), altona Diagnostics GmbH developed a real-time RT-PCR kit for the detection of MERS-CoV in patient samples.

The RealStar® MERS-CoV RT-PCR Kit 1.0 is the only CE-IVD marked *in vitro* diagnostic kit available on the market.

Figure 1: Probit analysis for *upE* gene and *Orf1a* RT-PCR assays included in the RealStar® MERS-CoV RT-PCR Kit 1.0. The limits of detection for the *upE* and *Orf1a* assay were determined using *in-vitro* transcribed RNA (IVT) quantified by spectrophotometry. The IVT was diluted in half-logarithmic steps (from 100 to 0.03 copies/reaction) and tested in replicates (n=13) for positive amplification and detection. The *Orf1a* assay detects 9.25 copies/reaction with 95% probability (95% confidence interval (CI): 7 – 14 copies/reaction); the *upE*-assay has got a 95% cut-off value of 5.35 copies/reaction (95% CI: 4 – 9.7 copies/reaction).

Pathogen species present in sample	No. of samples	Specific upE / Orf1a RT-PCR signal	Internal Control upE / Orf1a
Enterovirus	3	-/-	+/+
Rhinovirus	5	-/-	+/+
Parainfluenza virus 1	2	-/-	+/+
Parainfluenza virus 2	3	-/-	+/+
Parainfluenza virus 3	2	-/-	+/+
Parainfluenza virus 4	2	-/-	+/+
Respiratory syncytial virus	5	-/-	+/+
Human metapneumovirus	2	-/-	+/+
Human Coronavirus (hCoV) NL63	2	-/-	+/+
hCoV OC43	3	-/-	+/+
hCoV 229E	2	-/-	+/+
hCoV HKU-1	1	-/-	+/+
Influenza A virus	4	-/-	+/+
Influenza B virus	2	-/-	+/+

Table 1: Analytical specificity. Possible unspecific cross-reactivity of the RealStar® MERS-CoV RT-PCR Kit 1.0 was tested with patient samples previously confirmed positive for nucleic acids of the enlisted pathogens.

WHO requires two independent positive PCR results to confirm MERS-CoV cases. Therefore, the kit contains 48 reactions of an RT-PCR system targeting the *Orf1a* gene and another 48 reactions targeting a region upstream of the *E* gene *(upE)*. Study data published in 2014 (Corman *et al.*, 2014) shows the suitability for clinical testing **(1)**. The kit is highly sensitive (*Orf1a:* 9.25 and *upE:* 5.35 copies/reaction) and specific and therefore facilitates reliable identification of MERS-CoV cases.

1. Corman, V. M. *et al.* Performance and clinical validation of the RealStar® MERS-CoV Kit for detection of Middle East respiratory syndrome coronavirus RNA. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. (2014). Doi:10.1016/j.jcv.2014.03.012