altona DIAGNOSTICS

Instructions for Use

RealStar®
MERS-CoV RT-PCR Kit 1.0

01/2017 EN

RealStar®

RealStar®

MERS-CoV RT-PCR Kit 1.0

For use with

m2000rt (Abbott Diagnostics)

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

LightCycler® 480 Instrument II (Roche)

CE

IVD

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Content

1. Intended Use

The RealStar® MERS-CoV RT-PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of Middle East respiratory syndrome coronavirus (MERS-CoV) specific RNA.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A Target orf1a	4	60
Purple	Master B Target orf1a	4	120
Blue	Master A Target upE	4	60
Purple	Master B Target upE	4	120
Red	Positive Control	1	250
Green	Internal Control	1	1000
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® MERS-CoV RT-PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE	
i	***Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***
i	***It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).***

5. Background Information

In 2012, *Middle East Respiratory Syndrome coronavirus*, MERS-CoV (formerly named: human coronavirus Erasmus Medical Center, HCoV-EMC), was identified for the first time to cause severe illness in humans **[1, 2]**. Detection of the virus is preferably done in samples from the lower respiratory tract. Upper respiratory tract samples (swabs) showed lower virus detection rates **[3]**. World Health Organization (WHO) recommends the use of two independent PCR assays for confirmation of MERS-CoV cases **[4]**.

Various real-time RT-PCR assays have been published. Two assays, one targeting a region upstream of the E gene (upE) and the other targeting open reading frame 1a (orf1a), showed the highest sensitivity **[5,6]**. The RealStar® MERS-CoV RT-PCR Kit 1.0 was developed based on these two described assays.

[1] Bermingham A, Chand MA, Brown CS, Aarons E, Tong C, Langrish C, et al. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2012;17:20290.

[2] Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012;367:1814–20.

[3] Guery B, Poissy J, el Mansouf L, Séjourné C, Ettahar N, Lemaire X, et al. Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. Lancet 2013;381:2265–72.

[4] WHO. Laboratory Testing for Middle East Respiratory Syndrome Coronavirus 2013:http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_ Sept_2013.pdf.

[5] Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2012;17.

[6] Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2012;17.

NOTE	
i	***Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.***

6. Product Description

The RealStar® MERS-CoV RT-PCR Kit 1.0 is an *in vitro* diagnostic test system, based on real-time PCR technology, for the qualitative detection of Middle East respiratory syndrome coronavirus (MERS-CoV) specific RNA.

The RealStar® MERS-CoV RT-PCR Kit 1.0 consists of two independent assays, one targeting a region upstream of the E gene (*upE*) and the other targeting open reading frame 1a (*orf1a*) of the MERS-CoV genome. World Health Organization (WHO) recommends the use of two independent PCR assays for confirmation of MERS-CoV cases **[5]**.

Both assays include a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

In both assays, probes specific for MERS-CoV RNA are labeled with the fluorophore FAM™. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore JOE™. Using probes linked to distinguishable dyes enables the parallel detection of MERS-CoV specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides included in the two assays were previously published by Victor Corman et al. 2012a **[6]** and 2012 b **[7]**. One RT-PCR assay targets the *orf1a* (Master A with blue caps, corresponding Master B with purple caps) and the other targets a region upstream of the E-gene (*upE*) (Master A with blue caps, corresponding Master B with purple caps).

Following the WHO-case definition (http://www.who.int/), laboratory confirmation requires two positive RT-PCR results on independent targets. By parallel testing of samples with the *upE*- and the *orf1a*-assay, which are both included in the RealStar® MERS-CoV RT-PCR Kit 1.0, the WHO requirements for laboratory MERS-CoV case confirmation can be fulfilled.

Due to the molecular assembly and possible evolution of MERS-CoV, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results. By including two assays targeting two different regions of the genome, the risk is significantly reduced. In case only one of the two assays included in the kit gives a positive result, the sample should be retested. **Furthermore, positive samples should be sent to the national reference laboratory for confirmatory testing**.

Nevertheless, in case the circulating strains evolve and accumulate mutations an update of the primer/probe sets might be necessary.

The test consists of three processes in a single tube assay:

- Reverse transcription of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® MERS-CoV RT-PCR Kit 1.0 consists of:

- Four Master reagents
  - (Master A and Master B target *orf1a*)
  - (Master A and Master B target *upE*)
- Internal Control (IC)
- Positive Control
- PCR grade water

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and target detection of MERS-CoV specific RNA and Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® MERS-CoV RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- m2000rt (Abbott Diagnostics)
- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® MERS-CoV RT-PCR Kit 1.0.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® Viral RNA Mini Kit (QIAGEN)
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate.
The suitability of the nucleic acid extraction procedure for use with RealStar® MERS-CoV RT-PCR Kit 1.0 has to be validated by the user.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION	
	***If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.***
	***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® MERS-CoV RT-PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

► If the IC is used as a RT-PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A (*orf1a* or *upE*)	5 µl	60 µl
Master B (*orf1a* or *upE*)	10 µl	120 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**16 µl**	**192 µl**

► If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, add the IC during the nucleic acid extraction procedure.

► No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.

► If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A (*orf1a* or *upE*)	5 µl	60 µl
Master B (*orf1a* or *upE*)	10 µl	120 µl
Volume Master Mix	**15 µl**	**180 µl**

CAUTION	
	***If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.***

CAUTION	
	***No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.***

8.3 Reaction Setup

- ► Pipette 15 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	15 µl
Sample or Control	10 µl
Total Volume	**25 µl**

- ► Make sure that at least one Positive and one Negative Control is used per run.
- ► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the RealStar® MERS-CoV RT-PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

- ► Define the following settings:

Settings	
Reaction Volume	25 µl
Ramp Rate	Default
Passive Reference	None

9.2 Fluorescence Detectors (Dyes)

- ► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
MERS-CoV (*orf1a*) specific RNA	*orf1a*	FAM™	(None)
MERS-CoV (*upE*) specific RNA	*upE*	FAM™	(None)
Internal Control	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse Transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	58	00:45
			-	72	00:15

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® MERS-CoV RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

For a **valid** diagnostic test run, the following control conditions must be met:

Control ID	Detection Channel			
	FAM™ *upE*	JOE™ *upE*	FAM™ *orf1a*	JOE™ *orf1a*
Positive Control	+	+/-*	+	+/-*
Negative Control	-	+	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel				Result Interpretation
FAM™ *upE*	JOE™ *upE*	FAM™ *orf1a*	JOE™ *orf1a*	
+	+*	+	+*	MERS-CoV *upE* and *orf1a* specific RNA detected. Send positive samples to the national reference laboratory for confirmatory testing
+	+*	-	+	MERS-CoV *upE* specific RNA detected. No MERS-CoV *orf1a* specific RNA detected. Repeat testing. Send sample to the national reference laboratory for confirmatory testing.
-	+	+	+*	MERS-CoV *orf1a* specific RNA detected. No MERS-CoV *upE* specific RNA detected. Repeat testing. Send sample to the national reference laboratory for confirmatory testing.
-	+	-	+	Neither MERS-CoV *upE* nor MERS-CoV *orf1a* specific RNA detected. The sample does not contain detectable amounts of MERS-CoV specific RNA.
-	-	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high MERS-CoV RNA load in the sample can lead to a reduced or absent Internal Control signal.

11. Performance Evaluation

Performance evaluation of the RealStar® MERS-CoV RT-PCR Kit 1.0 was done in collaboration with the coronavirus experts from the group of Prof. Dr. Christian Drosten (Department of Virology, University of Bonn Medical Center, Bonn, Germany). Patient samples from an imported case to Munich, Germany, and MERS-CoV RNA extracted from cell-culture were used as characterized positive material. MERS-CoV *upE* and *orf1a* specific in vitro transcripts of known concentration were utilized for quantitative purposes.

11.1 Analytical Sensitivity

Analytical sensitivity of the RealStar® MERS-CoV RT-PCR Kit 1.0 is defined as the concentration (copies per µl of the eluate) of *orf1a* or *upE* specific RNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of *orf1a* and *upE* specific *in vitro* transcripts (IVT) of known concentration.

Table 1: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of orf1a specific RNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10.000	13	13	100
3.162	13	13	100
1.000	13	12	92
0.316	13	7	54
0.100	13	1	8
0.032	13	1	8
0.010	13	0	0
0.003	13	0	0

Table 2: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of upE specific RNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10.000	13	13	100
3.162	13	13	100
1.000	13	13	100
0.316	13	6	46
0.100	13	3	23
0.032	13	0	0
0.010	13	0	0
0.003	13	0	0

The analytical sensitivity of the RealStar® MERS-CoV RT-PCR Kit 1.0 was determined by Probit analysis:

- For the detection of *orf1a* specific RNA, the analytical sensitivity is 0.93 copies/µl [95% confidence interval (CI): 0.70-1.41 copies/µl]
- For the detection of *upE* specific RNA, the analytical sensitivity is 0.54 copies/µl [95% confidence interval (CI): 0.40-0.97 copies/µl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® MERS-CoV RT-PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant MERS-CoV genotypes will be detected.

The analytical specificity of the RealStar® MERS-CoV 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.

The RealStar® MERS-CoV RT-PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Coronavirus NL63
- Coronavirus OC43
- Coronavirus 229E
- Coronavirus HKU-1
- Influenza A virus H3N2
- Influenza A virus H1N1
- Influenza B virus
- Enterovirus 71
- Rhinovirus 16
- Human parainfluenza virus 1
- Human parainfluenza virus 2
- Human parainfluenza virus 3
- Human parainfluenza virus 4a/b
- Human respiratory syncytial virus A
- Human respiratory syncytial virus B
- Human metapneumovirus A
- Human metapneumovirus B

11.3 Precision

Precision of the RealStar® MERS-CoV RT-PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analysis.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (C_t) - values. At least six replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 3: Precision data for orf1a and upE specific RNA

orf1a and *upE*		Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	*orf1a*	28.96	0.08	0.29
	upE	29.35	0.08	0.28
Inter-Assay Variability	*orf1a*	28.69	0.31	1.08
	upE	29.43	0.12	0.40
Inter-Lot Variability	*orf1a*	28.49	0.14	0.49
	upE	29.14	0.39	1.34
Total Variability	*orf1a*	28.71	0.18	0.62
	upE	29.31	0.19	0.67

Table 4: Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	29.39	0.160	0.56
Inter-Assay Variability	29.80	0.558	1.87
Inter-Lot Variability	29.72	0.632	2.13
Total Variability	29.64	0.450	1.52

11.4 Diagnostic Evaluation

The diagnostic evaluation of the RealStar® MERS-CoV RT-PCR Kit 1.0 was performed at the Institute of Virology, University of Bonn Medical Centre, Bonn, Germany.

Nineteen different samples from a patient were analysed with in-house *upE* and *orf1a* specific assays and the *upE* and *orf1a* specific assays as part of the RealStar® MERS-CoV RT-PCR Kit 1.0. Among these, 8 were tested positive with the in-house assays and with the RealStar® MERS-CoV RT-PCR Kit 1.0; 7 showed a negative results with the *in-house* assays as well as with the RealStar® MERS-CoV RT-PCR Kit 1.0; 4 showed a discrepant result (3 samples were positive only with the RealStar® MERS-CoV RT-PCR Kit 1.0 and 1 was positive only with the *in-house* assays).
The discrepant results were all generated with samples with a very low virus load indicated by a high C_t-value, representing rather a statistical variation at the limit of detection than a real sensitivity difference.

Table 5: Patient samples tested with in-house MERS-CoV specific RT-PCR and RealStar® MERS-CoV RT-PCR Kit 1.0

Sample	Day after onset	*in-house* (*upE*)[1]	RealStar® MERS-CoV (*upE*)[1]	RealStar® MERS-CoV (*orf1a*)[1]
Aspiration tube, rinsed with PBS	16	40.00	40.00	37.74
Aspiration tube, filter piece	16	35.80	35.00	36.17
BAL	12	34.06	31.82	32.44
BAL	12	34.96	32.67	33.16
BAL	14	35.99	34.60	34.82
BAL	13	-	-	-
Exudate, mouth[2]	16	-	36.60	-
Exudate, mouth	16	-	-	-
Exudate, mouth	16	-	-	-
Exudate, nose[3]	16	38.38	-	-
Exudate, nose[2]	16	-	40.00	40.00
Exudate, mouth	16	-	-	-
Stool	12	38.98	31.69	31.78
Stool	12	39.37	40.00	-
Stool	16	40.00	40.00	-
Urine[2]	12	-	40.00	37.86
Urine (catheter)	12	-	-	-
Urine (catheter)	13	-	-	-
Central venous catheter, rinsed tube	12	-	-	-

[1] numbers in columns indicate the respective C_t of positive real-time RT-PCR runs

[2] positive result only with commercial assay

[3] positive result only with in-house assay

12. Limitations

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the MERS-CoV genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogen.
- As with any diagnostic test, results of the RealStar® MERS-CoV RT-PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® MERS-CoV RT-PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For technical advice, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C.,Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

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The RealStar® MERS-CoV RT-PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

17. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade identification number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

RealStar® MERS-CoV RT-PCR Kit 1.0

always a drop ahead.

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