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RealStar®
MERS-CoV RT-PCR Kit U.S.
Instructions for Use

For Use Under the Emergency Use Authorization (EUA) Only

Version 02/2016

always a drop ahead.

RealStar®
MERS-CoV
RT-PCR Kit U.S.

For use with

VERSANT® kPCR Molecular System AD (Siemens)

ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)

LightCycler® 480 Instrument II (Roche)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN)

CFX96™ system/Dx real-time system (BIO-RAD)

CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD)

For Use Under the Emergency Use Authorization (EUA) Only

IVD For *in vitro* diagnostic use

EUA For use only under Emergency Use Authorization

REF Product No.: 391022

Σ 48 rxns

Store at -25°C ... -15°C

October 2015

altona Diagnostics GmbH • Mörkenstraße 12 • D-22767 Hamburg

MAN-391020-EN-02

Content

RealStar® MERS-CoV RT-PCR Kit U.S.

1. Intended Use

The RealStar® MERS-CoV RT-PCR Kit U.S. is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of RNA from Middle East respiratory syndrome coronavirus (MERS-CoV):

- in lower respiratory samples (tracheal aspirate / tracheal secretions) from patients with signs and symptoms of Middle East respiratory syndrome coronavirus infection in conjunction with epidemiological risk factors.
- in nasopharyngeal swabs from asymptomatic individuals suspected of exposure to MERS-CoV cases based on epidemiological risk factors (e.g., contact with a probable or confirmed MERS-CoV case, history of travel to geographic locations where MERS-CoV cases were detected, or other epidemiologic links for which MERS-CoV testing may be indicated).

Testing with the RealStar® MERS-CoV RT-PCR Kit U.S. should not be performed unless the patient or patient contact meets clinical and/or epidemiologic criteria for testing suspect specimens.

Results are for the presumptive identification of MERS-CoV. The definitive identification of MERS-CoV requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of MERS-CoV infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of Middle East respiratory syndrome coronavirus.

Negative results do not preclude Middle East respiratory syndrome coronavirus infection and should not be used as the sole basis for patient management decisions. The level of Middle East respiratory syndrome coronavirus that would be present in respiratory specimens from individuals with early infection is unknown.

The RealStar® MERS-CoV RT-PCR Kit U.S. is for use only under the MERS-CoV Emergency Use Authorization (EUA) by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories, and is limited to clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

2. Kit Components

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

3. Storage

- The RealStar® MERS-CoV RT-PCR Kit U.S. 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 at -25°C to -15°C upon arrival.
- Always check the expiration date and do not use reagents beyond the expiration date.
- 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 at +4°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 (chapter 6. Product Description):
  - ABI Prism® 7500 SDS (Applied Biosystems, Cat. No. 4351104)
  - ABI Prism® 7500 Fast SDS (Applied Biosystems, Cat. No. 4351106)
  - LightCycler® 480 Instrument II (Roche, Cat. No. 05015278001)
  - CFX96™ system/Dx real-time system (BIO-RAD, Cat. No. 185-5195)
  - CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD, Cat. No. 185-4095)
  - Rotor-Gene® 6000 (Corbett Research, Cat. No. P/N 6500, P/N 65H0, P/N 6600)
  - Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN, Cat. No. 9001570, 9002035)
  - VERSANT® kPCR Molecular System AD (Siemens, Cat. No. 10282939)
- Universal Transport Medium, UTM™ (Copan, Murrieta, California, USA) for processing of nasopharyngeal swab specimens.
- Appropriate nucleic acid extraction system or kit:
  - QIAamp® Viral RNA Mini Kit (QIAGEN, Cat. No. 52906 or 52904)
- Desktop centrifuge with a rotor for 2 ml reaction tubes (Eppendorf 5415C or equivalent)
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer (VWR 58810-163 or equivalent)
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)
- Nuclease-Free Water (not DEPC-Treated), Life Technologies (Cat. No. 4387936) or equivalent
- 10x PBS (Sigma-Aldrich, Cat. No. P5493) or equivalent

RealStar® MERS-CoV RT-PCR Kit U.S.

NOTE

> ⚠ ***Please ensure that instruments have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***

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]. As of June 16th 2015 there have been more than 150 confirmed cases of MERS-CoV in South-Korea and China and more than 1000 cases in Saudi-Arabia [3]. Detection of the virus is preferably done in samples from the lower respiratory tract. Upper respiratory tract samples (swabs) showed lower virus detection rates [4]. World Health Organization (WHO) recommends the use of two independent PCR assays for confirmation of MERS-CoV cases [5].

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 [6, 7]. The RealStar® MERS-CoV RT-PCR Kit U.S. 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] WHO. WHO | Middle East respiratory syndrome coronavirus (MERS-CoV) summary and literature update – as of 20 September 2013 2013:http://www.who.int/csr/disease/coronavirus_infections.

[4] 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.

[5] 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.

[6] 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.

[7] 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

> ⚠ ***Due to the molecular evolution of coronaviruses, there is an inherent risk for any PCR based test system that accumulation of mutations over time may lead to false negative results.***

6. Product Description

1. The RealStar® MERS-CoV RT-PCR Kit U.S. 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 U.S. 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. 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.

   The test is based on real-time RT-PCR technology, utilizing 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 labeled 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.

2. Workflow

a) Lower respiratory samples

The workflow starts with taking a lower respiratory sample (e.g. tracheal aspirate). Further processing of the specimen must take place following appropriate CDC guidelines, *Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV).* For nucleic acid extraction the QIAamp® Viral RNA Mini Kit (QIAGEN, Hilden, Germany) must be used. Nucleic acid extraction starts with a pre-incubation step for the sample not described in the QIAamp® Viral RNA Mini Kit manual. For this, 80 µl of tracheal aspirate / tracheal secretion is mixed with 20 µl of Proteinase

K, 120 µl PBS, and 200 µl AL buffer, and incubated for 10 minutes at 56 °C. Next, following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN), 140 µl of the mix is used for the extraction of the RNA. Elution is performed with 200 µl AVE buffer. The eluate contains the extracted RNA which will serve as template for analysis with the RealStar® MERS-CoV RT-PCR Kit U.S.

b) Nasopharyngeal swabs
The workflow starts with collecting a nasopharyngeal swab and placing this in Universal Transport Medium, UTM™ (Copan, Murrieta, California, USA). Further processing of the specimen has to take place under BSL2 conditions or in a Class II BSC until the sample is completely inactivated. For nucleic acid extraction the QIAamp® Viral RNA Mini Kit (QIAGEN, Hilden, Germany) must be used. Following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN), 140 µl of the swab wash are used for the extraction of the RNA. Elution is performed with 60 µl AVE buffer. The eluate contains the extracted RNA which will serve as template for analysis with the RealStar® MERS-CoV RT-PCR Kit U.S.

The temperature cycling and signal detection can be done with the real-time PCR cyclers listed as followed:

- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- CFX96™ system/Dx real-time system (BIO-RAD)
- CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN)
- VERSANT® kPCR Molecular System AD (Siemens)

The evaluation of the results and positive or negative calling of the samples is the last step of the workflow.

3. The RealStar® MERS-CoV RT-PCR Kit U.S. consists of:

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

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

4. Control material to be used with the RealStar® MERS-CoV RT-PCR Kit U.S. includes:

a) Internal Control (IC)
The Internal Control contains a defined copy number of an "artificial" RNA molecule with no homologies to any other known sequences. It has to be added to the nucleic acid extraction procedure and is reverse transcribed, amplified and detected in parallel to the MERS-CoV specific RNA. The function of the Internal Control is to ensure the integrity of MERS-CoV specific real-time RT-PCR results by indicating potential RT-PCR inhibition.

b) PCR grade water
The PCR grade water is to be used as negative control for the RT-PCR reaction. Its function is to indicate contamination of RT-PCR reagents.

c) Positive Control
The Positive Control consists of dilutions of a 1:1 mixture of two *in vitro* transcripts (IVT). One IVT contains a sequence of open reading frame 1a of MERS-CoV, whereas the other IVT contains a sequence upstream of the E gene of the MERS-CoV genome. The *orf1a* specific IVT as well as the *upE* specific IVT contain the target region for the *orf1a* and the *upE* specific detection system,

respectively, which is used to detect MERS-CoV specific RNA with the RealStar® MERS-CoV RT-PCR Kit U.S.. The Positive Control is used for the RT-PCR to verify the functionality of the MERS-CoV RNA specific RT-PCR detection system, which is included in the RealStar® MERS-CoV RT-PCR Kit U.S..

d) Negative Process Control (NPC)
Apart from the controls provided with the RealStar® MERS-CoV RT-PCR Kit U.S. a water sample (Nuclease-Free Water (not DEPC-Treated), Life Technologies (Cat. No. 4387936) or equivalent) or a 10x PBS sample (Sigma-Aldrich, Cat. No. P5493) or equivalent) should be included in each run as Negative Process Control (NPC) to monitor the nucleic acid extraction procedure.

7. Test Principle

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

- Reverse transcription of target RNA 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 U.S. is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The MERS-CoV specific primer and probe set is designed to detect RNA from the MERS-CoV in lower respiratory samples (tracheal aspirate / tracheal secretions) from patients presenting signs and symptoms of the MERS-CoV infection as well as in nasopharyngeal swabs from asymptomatic patient contacts in conjunction with epidemiological risk factors.

One-step RT-PCR assays are one-tube assays that first reverse-transcribe specific RNA templates into cDNA copies. This cDNA then undergoes a polymerase chain reaction (PCR) that utilizes a thermocyclic heating and cooling of the reaction to logarithmically amplify a specific region of DNA. The probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle.

8. Limitations and Precautions

- Negative results do not preclude infection with MERS-CoV and should not be used as the sole basis of a patient treatment/management decision. All results should be interpreted by a trained professional in conjunction with review of the patient's history and clinical signs and symptoms.
- Performance of nasopharyngeal swabs from symptomatic patients has not been established. Therefore, this assay should not be used with nasopharyngeal swabs from symptomatic patients.
- This test has not been evaluated for and should not be used with sample types other than tracheal aspirate/tracheal secretions from symptomatic patients and nasopharyngeal swabs from asymptomatic contacts.
- 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. False positive results may occur from cross-contamination by target organisms, their nucleic acids or amplified product.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. Improper collection, storage, or transport of specimens may lead to false negative results.
- The impact of the administration of MERS-CoV therapeutics on the ability to detect MERS-CoV RNA in patient specimens has not been evaluated.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction using the QIAamp® Viral RNA Mini Kit must be conducted prior to using this assay.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the virus genome covered by the primer and/or probes of the test may result in failure to detect the presence of the pathogen.

9. Warnings and Precautions

- This assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization only.
- Local, state, and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Middle East Respiratory Syndrome. Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive detection OR no detection (negative) MERS-CoV test result on the need for additional testing and appropriate transportation of specimens.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Use of this product is limited to specified laboratories and clinical laboratory personnel who have been trained on authorized instruments.
- Results need to be interpreted in conjunction with clinical signs, symptoms and travel history of the patient or contact information.
- Do not use reagents from other manufacturers with this assay.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen 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) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering different areas.
- 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 use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.
- Performance of the RealStar® MERS-CoV RT-PCR Kit U.S. has only been evaluated for tracheal aspirate / tracheal secretion specimens and nasopharyngeal swabs in conjunction with the QIAamp® Viral RNA Mini Kit.
- 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.

10. Instructions for Use

10.1 RNA Extraction using the QIAamp® Viral RNA Mini Kit

Details on the RNA extraction process using the QIAamp® Viral RNA Mini Kit (QIAGEN) are given below. Carefully read the manufacturer's instructions for use (QIAamp® Viral RNA Mini Handbook 04/2010) for general handling instructions.

10.1.1 Addition of carrier RNA and Internal Control template to Buffer AVL

Check Buffer AVL for precipitate, and if necessary incubate at 80°C until precipitate is dissolved. Prepare the required amount of Buffer AVL freshly. Calculate the volume of "Buffer AVL/carrier RNA/Internal Control template"-mix needed per batch of samples by selecting the number of samples to be simultaneously processed from Table 1. Mix the reagents by inverting the tube 10 times. The number of samples is defined by the number of patient samples to be tested plus one additional Negative Process Control (NPC; Nuclease-Free Water (not DEPC-Treated), Life Technologies (Cat. No. 4387936) or 10x PBS (Sigma-Aldrich, Cat. No. P5493) or equivalent).

Table 1: Volumes of Buffer AVL, carrier RNA and Internal Control template required for the QIAMP® Viral RNA Mini Kit procedure

No. of Samples	Vol. of Buffer AVL[a] (ml)	Vol. of carrier RNA[a] (µl)	Vol. of IC template (green lid)[b] (µl)
1	0.56	5.60	20.00
2	1.12	11.20	40.00
3	1.68	16.80	60.00
4	2.24	22.40	80.00
5	2.80	28.00	100.00
6	3.36	33.60	120.00
7	3.92	39.20	140.00
8	4.48	44.80	160.00
9	5.04	50.40	180.00
10	5.60	56.00	200.00
11	6.16	61.60	220.00
12	6.72	67.20	240.00
13	7.28	72.80	260.00
14	7.84	78.40	280.00
15	8.40	84.00	300.00
16	8.96	89.60	320.00
17	9.52	95.20	340.00
18	10.08	100.80	360.00
19	10.64	106.40	380.00
20	11.20	112.00	400.00
21	11.76	117.60	420.00
22	12.32	123.20	440.00
23	12.88	128.80	460.00
24	13.44	134.40	480.00

[a] supplied with the QIAamp® Viral RNA Mini Kit

[b] supplied with the RealStar® MERS-CoV RT-PCR Kit U.S.

Do not forget to reconstitute buffers AW1 and AW2 with 96-100% ethanol (see manufacturer guidelines for more information).

10.1.2 Extraction Procedure

10.1.2.1 Extraction from Lower Respiratory Samples

1. Mix 80 µl of a tracheal aspirate / tracheal secretion with 20 µl of Proteinase K, 120 µl 10xPBS, 200 µl AL buffer, pulse-vortex for 15 seconds and incubate for 10 minutes at 56 °C. For Negative Process Control (NPC), 80 µl of water or 10xPBS is used instead of specimen.
2. Pipette 560 µl of prepared Buffer AVL containing carrier RNA and Internal Control template into a 2 ml labelled microcentrifuge tube.
3. Add 140 µl of the pre-incubation mix containing the specimen (see step 1) or 140 µl of the pre-incubation mix containing water or 10x PBS for NPC to be extracted to the 2 ml labelled RNase-free microcentrifuge tube and mix by pulse-vortexing for 15 seconds.
4. Incubate specimen(s) and control at room temperature (15–25°C) for 10 minutes.
5. Briefly centrifuge the tubes to remove drops from the inside of the lid.
6. Add 560 µl of 96-100% ethanol to each specimen and control tube, and mix by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the tubes to remove drops from inside the lid.
7. For each specimen and control, place a QIAamp® spin column into a 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit). Be sure to label the top of the columns clearly.
8. Carefully transfer 630 µl of the mixture from step 6 to the QIAamp® spin column WITHOUT moistening the rim of the column.
9. Centrifuge 1 minute at 6,000 x g. If the specimen has not cleared the filter after the first run, repeat centrifugation until the specimen has cleared the filter. Discard the collection tube containing the flow through.

10. For each specimen and control, place the QIAamp® spin column into a second, clean 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit). Add the remaining mixture from step 6 to the respective spin column WITHOUT moistening the rim of the column. Pay special attention to add the remaining mixture to the correct column!
11. Centrifuge 1 minute at 6,000 x g. If the specimen has not cleared the filter after the first run, repeat centrifugation until the specimen has cleared the filter. Discard the collection tube containing the flow through.
12. For each specimen and control, place the QIAamp® spin column into another, clean 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit) and add 500 µl of Buffer AW1. Discard the tube containing the filtrate from the previous step.
13. Centrifuge 1 minute at 6,000 x g. If the buffer has not cleared the filter after 1 minute, repeat centrifugation until buffer has cleared the filter.
14. Place each QIAamp® spin column into a fourth clean 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit). Carefully open the QIAamp® spin column and add 500 µl of Buffer AW2.
15. Centrifuge at full speed (approx. 14,000 x g) for 3 minutes. Discard the tube containing the filtrate from the previous step.
16. To eliminate any possible Buffer AW2 carryover, place the QIAamp® spin column into a new collection tube, discard the old collection tube, and centrifuge at full speed (approx. 14,000 x g) for 3 minutes.
17. Place the QIAamp® spin column in a clean, clearly labelled 1.5 ml RNase-free microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate.
18. Carefully open the QIAamp® spin column and add **200 µl** of Buffer AVE that has been equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 minute. Centrifuge at 6,000 x g for 1 minute, RNA is now present in the eluate and ready to test. Store extracted specimens and controls at 2-8°C until PCR master mixes are prepared.

Extracted specimens should be tested with the RealStar® MERS-CoV RT-PCR Kit U.S. within 6 hours of completing the extraction process. Residual unextracted specimens should be stored at 2-8°C while testing is in progress. Long-term storage of extracted specimens (>6 hours) should be at -20°C. Minimize (not to exceed 3) repeated freeze-thaw cycles.

10.1.2.2 Extraction from Nasopharyngeal Swabs

1. Pipette 560 µl of prepared Buffer AVL containing carrier RNA and Internal Control template into a 2 ml labelled microcentrifuge tube.
2. Add 140 µl of the swab wash or 10x PBS for NPC to be extracted to the 2 ml labelled RNase-free microcentrifuge tube and mix by pulse-vortexing for 15 seconds.
3. Incubate specimen(s) and control at room temperature (15–25°C) for 10 minutes.
4. Briefly centrifuge the tubes to remove drops from the inside of the lid.
5. Add 560 µl of 96-100% ethanol to each specimen and control tube, and mix by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the tubes to remove drops from inside the lid.
6. For each specimen and control, place a QIAamp® spin column into a 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit). Be sure to label the top of the columns clearly.
7. Carefully transfer 630 µl of the mixture from step 6 to the QIAamp® spin column WITHOUT moistening the rim of the column.
8. Centrifuge 1 minute at 6,000 x g. If the specimen has not cleared the filter after the first run, repeat centrifugation until the specimen has cleared the filter. Discard the collection tube containing the flow through.
9. For each specimen and control, place the QIAamp® spin column into a second, clean 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit). Add the remaining mixture from step 6 to the respective spin column WITHOUT

moistening the rim of the column. Pay special attention to add the remaining mixture to the correct column!

10. Centrifuge 1 minute at 6,000 x g. If the specimen has not cleared the filter after the first run, repeat centrifugation until the specimen has cleared the filter. Discard the collection tube containing the flow through.
11. For each specimen and control, place the QIAamp® spin column into another, clean 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit) and add 500 µl of Buffer AW1. Discard the tube containing the filtrate from the previous step.
12. Centrifuge 1 minute at 6,000 x g. If the buffer has not cleared the filter after 1 minute, repeat centrifugation until buffer has cleared the filter.
13. Place each QIAamp® spin column into a fourth clean 2 ml collection tube (from the QIAamp® Viral RNA Mini Kit). Carefully open the QIAamp® spin column and add 500 µl of Buffer AW2.
14. Centrifuge at full speed (approx. 14,000 x g) for 3 minutes. Discard the tube-containing the filtrate from the previous step.
15. To eliminate any possible Buffer AW2 carryover, place the QIAamp® spin column into a new collection tube, discard the old collection tube, and centrifuge at full speed (approx. 14,000 x g) for 3 minutes.
16. Place the QIAamp® spin column in a clean, clearly labelled 1.5 ml RNase-free microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate.
17. Carefully open the QIAamp® spin column and add **60 µl** of Buffer AVE that has been equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 minute. Centrifuge at 6,000 x g for 1 minute, RNA is now present in the eluate and ready to test. Store extracted specimens and controls at 2-8°C until PCR master mixes are prepared.

RealStar® MERS-CoV RT-PCR Kit U.S.

NOTES

⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

⚠ ***Ethanol is a strong inhibitor in real-time PCR. Make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone USA: **+1 415 777 1712**
phone headquarter Hamburg: **+49-(0)40-5480676-0**

10.2 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 U.S. 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, the Master Mix is set up according to the following pipetting scheme:

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

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

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

NOTE

> ⚠ ***Never add the Internal Control directly to the specimen!***

10.3 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).
- Make sure that each of the Positive Controls and at least one Negative Control is used per run.
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film, 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).

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

11. Programming of Real-Time PCR Instruments

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® MERS-CoV RT-PCR Kit U.S. on the LightCycler® 480 Instrument II (Roche), CFX96™ system/Dx real-time system (BIO-RAD), CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD), ABI Prism® 7500 SDS and ABI Prism® 7500 Fast SDS (Applied Biosystems), Rotor-Gene® 6000 (Corbett Research), Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN) and VERSANT® kPCR Molecular System AD (Siemens), please refer to chapter 11.4 “Special remarks on the setup of authorized cyclers”. For further questions please contact our Technical Support (see section 16).

11.1 Settings

- Define the following settings:

Settings	
Reaction Volume	25 µl
Ramp Rate	Default
Passive Reference	None

11.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes) or targets, respectively:

Detection	Detector Name	Reporter	Quencher
MERS-CoV specific RNA (*orf1a*)	*orf1a*	FAM	(None)
MERS-CoV specific RNA (*upE*)	*upE*	FAM	(None)
Internal Control	IC	JOE (HEX or VIC)	(None)

11.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse Transcription	Hold	1	-	55 °C	20:00 min
Denaturation	Hold	1	-	95 °C	2:00 min
Amplification	Cycling	45	-	95 °C	0:15 min
			√	58 °C	0:45 min
			-	72 °C	0:15 min

11.4 Special remarks on the setup of authorized cyclers

Please find below special remarks on the setup of LightCycler® 480 Instrument II (Roche), CFX96™ system/Dx real-time system (BIO-RAD), CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD), ABI Prism® 7500 SDS and ABI Prism® 7500 Fast SDS (Applied Biosystems), Rotor-Gene® 6000 (Corbett Research), Rotor-Gene® Q 5/6 plex Platform (QIAGEN) and VERSANT® kPCR Molecular System AD (Siemens).

11.4.1 Special remarks on the setup of LightCycler® 480 Instrument II

1. In the “Experiment settings”, select “Detection Format: Dual Color Hydrolysis Probe / UPL Probe”.
2. Make sure by checking the “Customize” field that the setting shown for the “Filter Combinations” are “FAM (465-510)” and “VIC/HEX/Yellow555 (533-580)”.

11.4.2 Special remarks on the setup of ABI Prism® 7500 SDS

Go to “Plate Setup”, “Define Targets and Samples”, “Assign Targets and Samples”:

1. Select the whole plate.
2. Click the assign-boxes for both targets. The targets should appear in the wells in the plate layout.
3. Make sure to choose “none” in the “Select the dye to use as the passive reference” (default setting is “ROX”).

11.4.3 Special remarks on the setup of ABI Prism® 7500 SDS Fast

The same settings for “Plate Setup” as for the ABI Prism® 7500 SDS apply (see above). For the Fast version, go to “Experiment properties”. The ramp speed has to be set to “Standard (~2 hours to complete a run)”. The RealStar® MERS-CoV RT-PCR Kit U.S. is not compatible with the fast cycling conditions and the increased ramp rates.

11.4.4 Special remarks on the setup of CFX96™ system/Dx real-time system and CFX96 Touch™ Deep Well Real-Time PCR Detection System

Open the “Plate Editor” window and select all wells of the 96 well-plate. Click “Select Fluorophores”. For “Channel 1” check the box behind FAM and for “Channel 2” check the box behind VIC. Assign samples to the wells by selecting the appropriate “Sample Type “and afterwards “Load” FAM and VIC to the wells. The target name of FAM should be set to “upE” and “orf1a”, respectively and the target name of VIC to “Internal Control”.

11.4.5. Special remarks on the setup of Rotor-Gene® 6000 and Rotor-Gene® Q 5/6 plex/MDx Platform

Chose the 72-Well-Rotor and the appropriate reaction volume. The Gain optimization should be performed before 1st acquisition.

11.4.6. Special remarks on the setup of VERSANT® kPCR Molecular System AD

Assign well type “Unknown” to the wells of the plate and check boxes FAM and JOE in the “collect fluorescence data” area. Reference dye has to be “none”.

12. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument.
For questions regarding data analysis of the RealStar® MERS-CoV RT-PCR Kit U.S. on authorized real-time PCR instruments please contact our Technical Support (see section 16).

12.1 Validity of Diagnostic Test Runs

12.1.1 Valid Diagnostic Test Run

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

Control ID	RealStar® MERS-CoV RT-PCR Kit U.S.			
	upE Assay		*orf1a* Assay	
	FAM Detection Channel	JOE/VIC Detection Channel (Internal Control)	FAM Detection Channel	JOE/VIC Detection Channel (Internal Control)
Positive Control	POSITIVE	POSITIVE	POSITIVE	POSITIVE
Negative Control (PCR grade water)	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
Negative Process Control (NCP)	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE

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

If a test run is **repeatedly invalid** please contact our Technical Support (see section 16).

12.2 Interpretation of Results

Sample ID	RealStar® MERS-CoV RT-PCR Kit U.S.				
	upE Assay		*orf1a* Assay		
	FAM Detection Channel	JOE/VIC Detection Channel (Internal Control)	FAM Detection Channel	JOE/VIC Detection Channel (Internal Control)	Result Interpretation
A	POSITIVE	POSITIVE*	POSITIVE	POSITIVE*	MERS-CoV *upE* and *orf1a* specific RNA detected. Positive specimens should be sent to the appropriate public health authority for confirmatory testing.
B	POSITIVE	POSITIVE*	NEGATIVE	POSITIVE*	MERS-CoV *upE* specific RNA detected. MERS-CoV *orf1a* specific RNA not detected. Repeat testing from original sample or collect and test a new sample.

Sample ID	RealStar® MERS-CoV RT-PCR Kit U.S.				
	upE Assay		*orf1a* Assay		Result Interpretation
	FAM Detection Channel	JOE/VIC Detection Channel (Internal Control)	FAM Detection Channel	JOE/VIC Detection Channel (Internal Control)	
C	NEGATIVE	POSITIVE*	POSITIVE	POSITIVE*	MERS-CoV *orf1a* specific RNA detected. MERS-CoV *upE* specific RNA not detected. Repeat testing from original sample or collect and test a new sample.
D	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	Neither MERS-CoV *upE* nor MERS-CoV *orf1a* specific RNA detected. The sample does not contain detectable amounts of MERS-CoV specific RNA.
E	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	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/VIC detection channel is not required for positive results in the FAM detection channel. A high MERS-CoV load in the sample can lead to reduced or absent Internal Control signals.

13. Analytical Performance Evaluation

13.1 Analytical Sensitivity

13.1.1 Analytical Sensitivity - Lower Respiratory Samples

Estimation of the Limit of Detection (LoD): Serial dilutions of RNA purified from MERS-CoV cell culture supernatant (stock concentration 3.25E+07 PFU/ml) obtained from the Department of Virology, University of Bonn (Germany) were prepared. The ratio between infectious particles and genome equivalents (geq) was determined using qRT-PCR and quantified *in vitro* transcribed RNA. One PFU of this particular virus preparation corresponds to 57.3 geq.

For extraction, 80 µl of a tracheal aspirate / tracheal secretion pool were mixed with 20 µl of Proteinase K, 120 µl 10x PBS, 200 µl AL buffer, mixed by pulse-vortexing for 15 seconds and incubated for 10 minutes at 56°C. After that, 140 µl of the mix were combined with 560 µl AVL buffer, spiked with 10 µl RNA dilution. The final mix was subjected to the extraction procedure following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN). Elution was performed in 200 µl AVE buffer. Each dilution was extracted in triplicate and tested with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96™/Dx Real-Time System (BIO-RAD). The lowest concentration at which all replicates tested positive was treated as the estimated LoD. The results can be found in the tables below:

Table 1: LoD estimate for the RealStar® MERS-CoV RT-PCR Kit U.S. (Target *upE*)

Target	Concentration PFU/ml	Call rate	Replicate 1 (Cp)	Replicate 2 (Cp)	Replicate 3 (Cp)
upE	16,957	3/3	29.24	29.17	29.15
	1,695.7	3/3	32.18	32.31	31.95
	169.57	3/3	37.26	38.76	35.74
	16.957	2/3	39.18	42.30	-
	1.6957	0/3	-	-	-
	0.16957	0/3	-	-	-

Table 2: LoD estimate for the RealStar® MERS-CoV RT-PCR Kit U.S. (Target *orf1a*)

Target	Concentration PFU/ml	Call rate	Replicate 1 (Cp)	Replicate 2 (Cp)	Replicate 3 (Cp)
orf1a	16,957	3/3	29.11	29.15	29.10
	1,695.7	3/3	32.39	32.15	32.30
	169.57	3/3	34.97	36.29	35.89
	16.957	0/3	-	-	-
	1.6957	1/3	-	-	38.63
	0.16957	0/3	-	-	-

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™/Dx Real-Time System detected 3/3 replicates at 169.57 PFU/ml for both, the *upE* and the *orf1a* target. The LoD will be confirmed at 169.57 PFU/ml.

Confirmation of the Limit of Detection (LoD): Based on the results obtained from the estimation of the LoD, a dilution of MERS-CoV RNA equivalent to 169.57 PFU/ml was prepared in AE buffer and then spiked into 20 individual human tracheal aspirate / tracheal secretion samples. Samples were prepared and pretreated as described above, extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) and tested with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96™/Dx Real-Time System (BIO-RAD). The results can be found in the table below:

Table 3: LoD confirmation on CFX96™/Dx Real-Time System

Virus concentration: 169.57 PFU/ml						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (VIC)	Pos/ Neg	Cp (FAM)	Cp (VIC)
1	+	35.09	29.78	+	34.85	30.04
2	+	36.57	29.45	+	33.52	29.73
3	+	35.89	30.33	+	35.05	30.29
4	+	35.16	28.94	+	33.97	28.81
5	+	36.30	29.69	+	34.69	29.86
6	+	35.87	28.95	+	34.30	28.89
7	+	36.75	29.28	+	34.99	29.20
8	+	36.13	29.12	+	34.05	28.99
9	-	N/D	30.60	+	35.57	30.78
10	+	37.42	29.08	+	34.19	29.15
11	+	38.32	28.94	+	34.94	29.05
12	+	41.26	29.81	+	35.60	29.70
13	+	35.95	29.33	+	36.01	29.45
14	+	36.01	29.05	+	34.18	29.34
15	+	36.62	28.98	+	34.17	29.23
16	+	38.29	29.27	+	34.59	29.38
17	+	36.60	29.33	+	35.39	29.27
18	+	40.00	31.03	+	36.48	30.66
19	+	35.88	28.90	+	35.21	29.07
20	+	37.99	29.27	+	35.80	29.19
Statistics	Mean Cp (n=19)	36.95	29.46	Mean Cp (n=20)	34.88	29.50
	SD	1.60	0.59	SD	0.77	0.57
	CV%	4.34	2.02	CV%	2.22	1.92
	Results	19/20		Results	20/20	

At the concentration of 169.57 PFU/ml at least 19/20 replicates were detected positive and thereby confirm the LoD to be 169.57 PFU/ml in human tracheal aspirate / tracheal secretion for the RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™/Dx Real-Time System (BIO-RAD).

***Confirmation of the Limit of Detection (LoD)* for additional real-time PCR instruments:** Based on the results obtained from the LoD confirmation using the CFX96™/Dx Real-Time System (BIO-RAD), a dilution of MERS-CoV RNA equivalent to 169.57 PFU/ml was prepared in AE buffer and then spiked into 20 individual human tracheal aspirate/tracheal secretion samples. Samples were prepared and pretreated as described above, extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) and tested with the RealStar® MERS-CoV RT-PCR Kit U.S. on the following additional real-time PCR Cycler systems:

1. ABI Prism® 7500 SDS (Applied Biosystems)
2. CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD)
3. Rotor-Gene® 6000 (Corbett Research)
4. VERSANT® kPCR Molecular System AD (Siemens)
5. LightCycler® 480 Instrument II (Roche)

The results can be found in the tables below:

Table 4: LoD confirmation on ABI Prism® 7500 SDS

Virus concentration: 169.57 PFU/ml						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (JOE)	Pos/ Neg	Cp (FAM)	Cp (JOE)
1	+	34.46	29.78	+	35.39	29.93
2	+	35.95	29.91	+	33.92	29.83
3	+	37.32	30.71	+	36.43	30.3
4	+	35.16	29.32	+	34.77	29.15
5	+	35.48	30.27	+	34.42	29.94
6	+	36.46	29.49	+	35.2	29.48
7	+	37.46	29.62	+	35.04	29.36
8	+	34.85	29.49	+	34.92	29.61
9	-	37.48	30.01	+	37.18	30.88
10	+	35.08	29.5	+	34.1	29.39
11	+	37.69	29.6	+	34.63	29.44
12	+	37.82	30.15	+	35.24	30.24
13	+	37.05	30.06	+	37.22	29.77
14	+	38.16	29.72	+	36.5	29.4
15	+	35.63	29.63	+	34.74	29.36
16	+	35.6	29.76	+	34.67	29.26
17	+	35.43	29.72	+	35.73	29.47
18	+	37.21	31.49	+	36.53	31.15
19	+	40.59	29.81	+	35.24	29.4
20	+	37.44	29.94	+	34.31	29.76
Statistics	Mean Cp (n=20)	36.62	29.90	Mean Cp (n=20)	35.31	29.76
	SD	1.48	0.49	SD	0.98	0.53
	CV%	4.03	1.65	CV%	2.79	1.79
	Results	20/20		Results	20/20	

At the concentration of 169.57 PFU/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 169.57 PFU/ml in human tracheal aspirate/tracheal secretion for the RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the ABI Prism® 7500 SDS (Applied Biosystems).

Table 5: LoD confirmation on CFX96 Touch™ Deep Well Real-Time PCR Detection System

Virus concentration: 169.57 PFU/ml						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (JOE)	Pos/ Neg	Cp (FAM)	Cp (JOE)
1	+	36.3	29.43	+	35.18	29.04
2	+	36.5	29.33	+	35.23	29.01
3	+	37.04	29.5	+	35.76	29.54
4	+	36.6	28.45	+	36.21	28.37
5	+	36.72	29.1	+	35.34	28.99
6	+	36.02	28.93	+	35.35	28.56
7	+	38.74	28.89	+	35.31	28.67
8	+	37.64	28.64	+	35.71	28.44
9	-	N/D	30.17	+	36.69	30.03
10	+	36.73	28.89	+	36.08	28.52
11	+	40.45	28.74	+	35.66	28.66
12	+	43.14	29.84	+	35.94	29.01
13	+	38.44	29.23	+	36.63	28.95
14	+	39.72	28.93	+	36.37	28.51
15	+	37.12	28.56	+	35.43	28.36
16	+	38.19	29.84	+	36.14	28.87
17	+	37.1	28.87	+	35.59	29.02
18	+	40.88	30.54	+	38.52	30.3
19	+	37.57	28.54	+	35.91	28.63
20	+	39.35	29.25	+	35.5	29.05
Statistics	Mean Cp (n=19)	38.12	29.18	Mean Cp (n=20)	35.93	28.93
	SD	1.87	0.57	SD	0.76	0.52
	CV%	4.91	1.94	CV%	2.11	1.79
	Results	19/20		Results	20/20	

At the concentration of 169.57 PFU/ml at least 19/20 replicates were detected positive and thereby confirm the LoD to be 169.57 PFU/ml in human tracheal aspirate/tracheal secretion for the RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD).

Table 6: LoD confirmation on Rotor-Gene® 6000

Virus concentration: 169.57 PFU/ml						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (JOE)	Pos/ Neg	Cp (FAM)	Cp (JOE)
1	+	34.43	27.43	+	33.83	27.48
2	+	34.02	27.43	+	32.66	27.2
3	+	34.59	27.51	+	32.85	27.5
4	+	33.17	26.99	+	31.48	26.62
5	+	36.63	27.36	+	32.48	27.43
6	+	33.83	26.9	+	32.78	26.83
7	+	35.04	26.91	+	32.06	26.9
8	+	33.11	26.9	+	31.95	26.79
9	+	38.04	28.38	+	33.7	28.3
10	+	35.18	26.91	+	32.27	26.57
11	+	34.67	26.85	+	32.25	26.78
12	+	34.52	27.49	+	33.2	27.15
13	+	36.41	27.33	+	32.5	26.89
14	+	38.27	26.92	+	32.66	26.9
15	+	34.55	26.92	+	32.07	26.48
16	+	34.41	26.97	+	31.64	26.7
17	+	34.02	26.99	+	33.08	27.02
18	+	38.83	28.19	+	34.36	28.63
19	+	34.64	26.74	+	32.72	26.68
20	+	35.52	27.19	+	32.53	26.88
Statistics	Mean Cp (n=20)	35.19	27.22	Mean Cp (n=20)	32.65	27.09
	SD	1.63	0.44	SD	0.72	0.56
	CV%	4.63	1.62	CV%	2.21	2.05
	Results	20/20		Results	20/20	

At the concentration of 169.57 PFU/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 169.57 PFU/ml in human tracheal aspirate/tracheal secretion for the RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the Rotor-Gene® 6000 (Corbett Research).

Table 7: LoD confirmation on VERSANT® kPCR Molecular System AD

Virus concentration: 169.57 PFU/ml						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (JOE)	Pos/ Neg	Cp (FAM)	Cp (JOE)
1	+	38.36	30.24	+	35.35	29.88
2	+	34.96	29.79	+	34.4	29.55
3	+	38.55	30.19	+	35.41	30.17
4	+	34.59	29.33	+	34.62	28.85
5	+	36.64	29.86	+	35.04	29.96
6	+	36.59	29.27	+	34.12	29.19
7	+	36.29	29.62	+	35.31	29.61
8	+	37.31	29.92	+	35.48	29.39
9	+	36.52	31.11	+	36.98	30.57
10	+	36.91	29.24	+	35.57	28.98
11	+	39.39	29.64	+	35.07	29.27
12	+	36.8	30.28	+	35.17	29.86
13	+	36.77	29.72	+	35.87	29.56
14	+	39.07	29.23	+	35.55	29.39
15	+	36.84	29.24	+	35.73	29.15
16	+	36.7	29.49	+	35.57	29.5
17	+	35.93	29.64	+	34.92	29.54
18	+	39.39	30.88	+	36.33	30.87
19	+	36.96	28.99	+	35.51	29.29
20	+	35.87	29.52	+	34.92	29.48
Statistics	Mean Cp (n=20)	37.02	29.76	Mean Cp (n=20)	35.35	29.60
	SD	1.33	0.55	SD	0.64	0.50
	CV%	3.60	1.86	CV%	1.81	1.70
	Results	20/20		Results	20/20	

At the concentration of 169.57 PFU/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 169.57 PFU/ml in human tracheal aspirate/tracheal secretion for the RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the VERSANT® kPCR Molecular System AD (Siemens).

Table 8: LoD confirmation on LightCycler® 480 Instrument II

Virus concentration: 169.57 PFU/ml						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (JOE)	Pos/ Neg	Cp (FAM)	Cp (JOE)
1	+	35.66	29.17	+	36.65	29.09
2	+	36.36	29.05	+	36.12	29.01
3	+	34.95	29.51	+	35.61	29.36
4	+	35.04	28.63	+	36.23	28.59
5	+	35.17	29.05	+	35.03	29.18
6	+	35.4	28.5	+	34.89	28.6
7	+	35.64	28.71	+	34.94	28.78
8	+	35.76	28.64	+	35.96	28.82
9	+	37.25	30.07	-	N/D	30.12
10	+	35.5	28.67	+	35.97	28.61
11	+	33.63	28.51	+	34.97	28.69
12	+	36.14	29.23	+	36.57	29.31
13	+	35.75	29.18	+	36.67	28.99
14	+	35.19	28.83	+	37.62	28.66
15	+	35.31	28.77	+	35.17	28.63
16	+	36.27	28.82	+	36.55	28.71
17	+	36.91	29	+	36.59	28.78
18	+	37.69	30.47	+	39.22	30.22
19	+	36.54	28.68	+	35.77	28.64
20	+	36.01	29	+	36.21	28.75
Statistics	Mean Cp (n=20)	35.81	29.02	Mean Cp (n=19)	36.14	28.98
	SD	0.90	0.50	SD	1.05	0.47
	CV%	2.52	1.74	CV%	2.90	1.63
	Results	20/20		Results	19/20	

At the concentration of 169.57 PFU/ml at least 19/20 replicates were detected positive and thereby confirm the LoD to be 169.57 PFU/ml in human tracheal aspirate/tracheal secretion for the RealStar® MERS-CoV RT-PCR Kit U.S.in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the LightCycler® 480 Instrument II (Roche).

13.1.2 Analytical Sensitivity - Nasopharyngeal Swabs

Estimation of the Limit of Detection (LoD): Serial dilutions of RNA purified from MERS-CoV cell culture supernatant (stock concentration 3.25E+07 PFU/mL) obtained from the Department of Virology, University of Bonn (Germany) were prepared. The ratio between infectious particles and genome equivalents (geq) was determined using qRT-PCR and quantified in vitro transcribed RNA. One PFU of this particular virus preparation corresponds to 57.3 geq.
For extraction, 140 µl of pooled nasopharyngeal swab washes were combined with 560 µL AVL buffer, containing 6 µl of the internal control template and spiked with 10 µL RNA dilution. The final mix was subjected to the extraction procedure following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN). Elution was performed in 60 µl AVE buffer. Each dilution was extracted in triplicate and tested with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96™/Dx Real-Time System (Bio-Rad). The lowest concentration at which all replicates tested positive was treated as the estimated LoD. The results can be found in the tables below:

Table 9: LoD estimate for the RealStar® MERS-CoV RT-PCR Kit U.S. on CFX96™/Dx Real-Time System (Target *upE*)

Target	Concentration PFU/ml	Call rate	Replicate 1 (Cp)	Replicate 2 (Cp)	Replicate 3 (Cp)
upE	1,748	3/3	29.25	29.10	29.11
	174.8	3/3	32.17	32.31	32.22
	55.2	3/3	33.55	34.35	34.07
	17.5	3/3	35.75	36.01	35.11
	1.8	2/3	-	41.98	41.36
	0.16957	0/3	-	-	-

Table 10: LoD estimate for the RealStar® MERS-CoV RT-PCR Kit U.S. on CFX96™/Dx Real-Time System (Target *orf1a*)

Target	Concentration PFU/ml	Call rate	Replicate 1 (Cp)	Replicate 2 (Cp)	Replicate 3 (Cp)
orf1a	1,748	3/3	28.80	28.43	28.78
	174.8	3/3	31.57	31.51	31.61
	55.2	3/3	33.00	33.61	33.14
	17.5	3/3	34.96	34.53	35.22
	1.8	3/3	37.34	37.33	38.44
	0.16957	0/3	-	-	-

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™/Dx Real-Time System detected 3/3 replicates at 17.5 PFU/mL for both, the *upE* and the *orf1a* target.

The extracts of samples containing 55.2 and 17.5 PFU/mL were tested on the Rotor-Gene® 6000 instrument to check whether the tentative LoD determined on the CFX96™/Dx Real-Time System would be the same using the Rotor-Gene® 6000. The results can be found in the tables below:

Table 11: LoD estimate for the RealStar® MERS-CoV RT-PCR Kit U.S. on Rotor-Gene® 6000 (Target *upE*)

Target	Concentration PFU/ml	Call rate	Replicate 1 (Cp)	Replicate 2 (Cp)	Replicate 3 (Cp)
upE	55.2	3/3	31.80	32.40	31.76
	17.5	2/3	-	34.35	34.54

Table 12: LoD estimate for the RealStar® MERS-CoV RT-PCR Kit U.S. on Rotor-Gene® 6000 (Target *orf1a*)

Target	Concentration PFU/ml	Call rate	Replicate 1 (Cp)	Replicate 2 (Cp)	Replicate 3 (Cp)
orf1a	55.2	3/3	30.25	30.67	30.76
	17.5	2/3	33.17	31.83	32.10

One replicate sample containing 17.5 PFU/mL gave a negative result on the Rotor-Gene® 6000 with the upE assay. Therefore, 55.2 PFU/mL was chosen as the tentative LoD value.

Confirmation of the Limit of Detection (LoD): Based on the results obtained from the estimation of the LoD, a dilution of MERS-CoV RNA equivalent to 55.2 PFU/mL was prepared in AE buffer and then spiked into 20 individual nasopharyngeal swab washes. Samples were prepared as described above, extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) and tested with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96™/Dx Real-Time System (Bio-Rad). The results can be found in the table below:

Table 13: LoD confirmation on CFX96™/Dx Real-Time System

Virus concentration = 55.2 PFU/mL						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (VIC)	Pos/ Neg	Cp (FAM)	Cp (VIC)
1	+	33.65	28.03	+	33.42	27.83
2	+	34.08	28.11	+	33.49	28.12
3	+	33.49	27.79	+	33.08	27.81
4	+	33.79	27.70	+	33.16	27.72
5	+	33.08	27.61	+	33.06	27.56
6	+	33.27	27.54	+	32.82	27.60
7	+	33.51	27.62	+	33.12	27.83
8	+	33.36	27.65	+	33.07	27.75
9	+	33.93	27.74	+	33.26	27.72
10	+	33.70	27.66	+	32.92	27.49
11	+	34.00	27.60	+	33.41	27.90
12	+	33.71	27.77	+	33.04	27.63
13	+	33.23	27.58	+	33.12	27.75
14	+	33.48	27.80	+	33.12	27.52
15	+	33.24	27.63	+	32.91	27.61
16	+	33.31	27.94	+	32.67	27.74
17	+	33.69	27.99	+	33.40	27.75
18	+	33.90	27.79	+	32.94	28.02
19	+	33.95	27.83	+	33.22	27.70
20	+	33.30	27.71	+	32.95	27.71
Statistics	Mean Cp (n=20)	33.58	27.75	Mean Cp (n=20)	33.11	27.74
	SD	0.29	0.16	SD	0.21	0.15
	CV%	0.86	0.58	CV%	0.63	0.54
	Results	20/20		Results	20/20	

At the concentration of 55.2 PFU/mL 20/20 replicates were detected positive and thereby confirm the LoD to be 55.2 PFU/mL in nasopharyngeal swab washes for the RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™/Dx Real-Time System (Bio-Rad).

Confirmation of the Limit of Detection (LoD) for the Rotor-Gene™ 6000 (Corbett Research): Based on the results obtained from the LoD confirmation using the CFX96™/Dx Real-Time System (Bio-Rad), a dilution of MERS-CoV RNA equivalent to 55.2 PFU/mL was prepared in AE buffer and then spiked into 20 individual human tracheal aspirate samples. Samples were prepared as described above, extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) and tested with the RealStar® MERS-CoV RT-PCR Kit U.S. on the Rotor-Gene® 6000 (Corbett Research). The results can be found in the tables below:

Table 14: LoD confirmation on Rotor-Gene® 6000

Virus concentration = 55.2 PFU/mL						
	upE			*orf1a*		
Specimen	Pos/ Neg	Cp (FAM)	Cp (VIC)	Pos/ Neg	Cp (FAM)	Cp (VIC)
1	+	34.24	27.67	+	31.61	27.99
2	+	34.80	28.11	+	32.23	27.69
3	+	34.00	27.76	+	31.74	28.05
4	+	33.80	27.90	+	31.79	27.79
5	+	34.76	27.72	+	31.47	27.81
6	+	34.03	27.87	+	31.66	27.85
7	+	35.07	27.62	+	31.30	27.63
8	+	33.81	27.64	+	31.66	27.89
9	+	34.36	27.59	+	32.21	27.66
10	+	34.61	27.82	+	32.35	27.92
11	+	34.62	27.81	+	31.62	27.87
12	+	34.39	28.11	+	31.80	27.81
13	+	34.47	27.51	+	31.28	27.75
14	+	34.03	27.62	+	31.34	27.77
15	+	34.28	27.60	+	31.52	27.61
16	+	33.26	27.77	+	31.44	27.47
17	+	35.46	27.67	+	31.90	27.65
18	+	34.19	27.68	+	31.70	27.73
19	+	34.83	27.75	+	31.64	27.85
20	+	35.38	28.17	+	31.50	27.62
Statistics	Mean Cp (n=20)	34.42	27.80	Mean Cp (n=20)	31.69	27.77
	SD	0.53	0.18	SD	0.23	0.14
	CV%	1.54	0.65	CV%	0.73	0.50
	Results	20/20		Results	20/20	

At the concentration of 55.2 PFU/mL 20/20 replicates were detected positive and thereby confirm the LoD to be 55.2 PFU/mL in nasopharyngeal swab washes for the RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the Rotor-Gene® 6000 (Corbett Research).

13.2 Analytical Specificity

13.2.1 Reactivity

The oligonucleotides for *upE* and *orf1a* detection target highly conserved regions of the MERS-CoV genome. Until now, no mismatch that would impair detection of MERS-CoV has been identified. Two sequences (NCBI Genbank, KJ156881 and KJ156873) have been identified that have a single mismatch each with the *upE* forward primer and the *upE* probe, respectively. These mismatches did not lead to lower RT-PCR performance as determined with *in vitro* transcribed RNA resembling these sequence phenotypes.

13.2.2 Cross-Reactivity

To evaluate the analytical specificity of the RealStar® MERS-CoV RT-PCR Kit U.S. with regards to cross-reactivity, genomic RNA or DNA from different relevant pathogens was tested. The genomic RNA or DNA was extracted from cell culture supernatant or preparations from different providers (ATCC or University of Bonn, Germany). All samples were analyzed with the RealStar® MERS-CoV RT-PCR Kit U.S., distributed across three different real-time PCR cycler systems (LightCycler® 480 Instrument II (Roche), Rotor-Gene® 6000 (Corbett Research), and CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD)).

Table 15: Analytical specificity of the RealStar® MERS-CoV RT-PCR Kit U.S. with regards to cross-reactivity

		RealStar® MERS-CoV RT-PCR Kit U.S.			
Pathogen	Strain	Result for MERS-CoV *upE*	Result for IC (*upE*)	Result for MERS-CoV *orf!a*	Result for IC (*orf1a*)
Adenovirus	Not available	Negative	Positive	Negative	Positive
Coronavirus	229E	Negative	Positive	Negative	Positive
Coronavirus	OC43	Negative	Positive	Negative	Positive
Coronavirus	HKU1	Negative	Positive	Negative	Positive
Coronavirus	NL63	Negative	Positive	Negative	Positive
Enterovirus 68	F02-3607 Corn	Negative	Positive	Negative	Positive
HMPV	Not available	Negative	Positive	Negative	Positive
Influenza A	H1N1nv	Negative	Positive	Negative	Positive
Influenza A	H3N2 (A/ Aichi/2/68)	Negative	Positive	Negative	Positive
Influenza B	B/Lee/40	Negative	Positive	Negative	Positive
Parainfluenza 1	C35	Negative	Positive	Negative	Positive
Parainfluenza 2	Greer	Negative	Positive	Negative	Positive
Parainfluenza 3	C 243	Negative	Positive	Negative	Positive
Parainfluenza 4a	M-25	Negative	Positive	Negative	Positive
Parainfluenza 4b	CH 19503	Negative	Positive	Negative	Positive
Respiratory syncytial virus A	Long	Negative	Positive	Negative	Positive
Rhinovirus 58	21-CV 20 (NIAID V-136-002-021)	Negative	Positive	Negative	Positive

Table 15: (continuation)

		RealStar® MERS-CoV RT-PCR Kit U.S.			
Pathogen	Strain	Result for MERS-CoV *upE*	Result for IC (*upE*)	Result for MERS-CoV *orf!a*	Result for IC (*orf1a*)
Bordetella pertussis	Not available	Negative	Positive	Negative	Positive
Chlamydia pneumonia	Not available	Negative	Positive	Negative	Positive
Escherichia coli	Not available	Negative	Positive	Negative	Positive
Haemophilus influenzae	Not available	Negative	Positive	Negative	Positive
Moraxella cararrhalis	Not available	Negative	Positive	Negative	Positive
Mycoplasma pneumoniae	M129-B7	Negative	Positive	Negative	Positive
Neisseria meningitidis	Not available	Negative	Positive	Negative	Positive
Pseudomonas aeruginosa	Not available	Negative	Positive	Negative	Positive
Staphylococcus aureus	Not available	Negative	Positive	Negative	Positive
Streptococcus pneumonia	Not available	Negative	Positive	Negative	Positive
Streptococcus pyogenes	Not available	Negative	Positive	Negative	Positive

No cross-reactivity of the RealStar® MERS-CoV RT-PCR Kit U.S. with genomic RNA or DNA of the selected pathogens was observed. All samples tested generated a positive Internal Control signal in the JOE/VIC channel, whereas no signal was observable in the target specific (FAM) channel for MERS-CoV *upE* and *orf1a*. All pathogens were tested at clinically relevant levels.

For additional pathogens with limited or no availability, an *in silico* analysis was done showing that cross-reactivity is unlikely to occur.

Table16: Pathogens checked for cross-reactivity of the RealStar® MERS-CoV RT-PCR Kit U.S. via *in silico* analysis

Pathogen
Coronavirus SARS (Urbani)
Influenza A H3N1 (A/Texas/12)
Parechovirus 1b
Candida albicans (yeast)
Corynebacterium diphtheriae
Lactobacillus plantarium
Legionella pneumophila
Mycobacterium tuberculosis
Neisseria elongata
Staphylococcus epidermis
Streptococcus salivarius

The primer sequences were analyzed using a search against selected species (viruses and bacteria) specified in the table above. The BLAST algorithms were set to: blastn; Max target sequences: 10,000; Expect threshold: 1,000; Word size 7; Match/mismatch scores: 1,-3; Gap Costs Existence: 5 Extension: 2.

All primers contained in the RealStar® MERS-CoV RT-PCR Kit U.S. were analyzed in all possible combinations against the sequences of indicated organisms. Hits were reviewed for potential formation of PCR product through binding of the primers in close proximity and with the right orientation to each other on target nucleic acid molecules. No constellation was found that could lead to undesired amplification of potentially cross-reacting target sequences.

14. Clinical Performance Evaluation

14.1 Mock clinical study - Lower Respiratory Samples

Data from the LoD study confirmed that in lower respiratory samples (tracheal aspirate / tracheal secretion) the LoD of the RealStar® MERS-CoV RT-PCR Kit U.S. for MERS-CoV is 169.57 PFU/ml. To predict clinical performance at the 95% confidence interval (CI), viral RNA at different concentrations was prepared, blinded and spiked into overall 45 samples of individual tracheal aspirate / tracheal secretion samples prepared and pretreated as described in section 10.1.2 Extraction Procedure. Fifteen specimens each were spiked at 2.25x LoD (382 PFU/ml), at 3x LoD (509 PFU/ml), and at a concentration of 200x LoD (33,914 PFU/ml). Another 55 individual human tracheal aspirate/tracheal secretion samples were left unspiked. All samples were blinded, handed to an unbiased operator and extracted using the QIAamp® Viral RNA Mini Kit (QIAGEN). Eluates were analyzed with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96™/Dx Real-Time System (BIO-RAD), LightCycler® 480 Instrument II (Roche), Rotor-Gene® 6000 (Corbett Research), ABI Prism® 7500 SDS (Applied Biosystems), VERSANT® kPCR Molecular System AD (Siemens), and CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD), respectively. The blinded spiking key was unmasked after the results were complete. The results of the analyses can be found in the tables below.

14.1.1 RealStar® MERS-CoV RT-PCR Kit U.S. on CFX96™/ Dx Real-Time System

The results of the analysis with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96™ /Dx Real-Time System (BIO-RAD) can be found in the tables below.

Table 17: Mock clinical study: RealStar® MERS-CoV RT-PCR Kit U.S. on CFX96™/Dx Real-Time System

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
1	-	N/A	-	-	29.24	YES	-	-	28.97	YES
2	-	N/A	-	-	29.34	YES	-	-	29.16	YES
3	200x LOD	33,914	29.76	+	29.31	YES	28.65	+	29.06	YES
4	-	N/A	-	-	29.23	YES	-	-	28.81	YES
5	-	N/A	-	-	29.20	YES	-	-	28.97	YES
6	-	N/A	-	-	29.24	YES	-	-	29.16	YES
7	-	N/A	-	-	29.19	YES	-	-	28.98	YES
8	3x LOD	509	35.48	+	29.21	YES	34.24	+	29.11	YES
9	2.25x LOD	382	37.00	+	29.25	YES	34.67	+	29.06	YES
10	3x LOD	509	35.67	+	29.07	YES	34.20	+	28.91	YES
11	3x LOD	509	35.56	+	29.41	YES	34.66	+	29.24	YES
12	-	N/A	-	-	29.10	YES	-	-	28.96	YES
13	200x LOD	33,914	29.75	+	29.18	YES	28.74	+	29.19	YES
14	-	N/A	-	-	28.96	YES	-	-	28.79	YES
15	-	N/A	-	-	29.16	YES	-	-	28.92	YES
16	200x LOD	33,914	29.60	+	29.25	YES	28.60	+	29.05	YES
17	-	N/A	-	-	29.13	YES	-	-	29.13	YES
18	2.25x LOD	382	35.80	+	29.33	YES	34.89	+	29.16	YES
19	2.25x LOD	382	36.19	+	29.21	YES	34.71	+	29.03	YES
20	-	N/A	-	-	29.23	YES	-	-	28.99	YES

Table 17: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
21	-	N/A	-	-	29.05	YES	-	-	28.90	YES
22	-	N/A	-	-	28.98	YES	-	-	28.99	YES
23	-	N/A	-	-	28.74	YES	-	-	28.82	YES
24	2.25x LOD	382	35.59	+	28.86	YES	34.24	+	28.70	YES
25	-	N/A	-	-	29.02	YES	-	-	28.71	YES
26	3x LOD	509	35.90	+	28.90	YES	34.37	+	28.87	YES
27	-	N/A	-	-	29.03	YES	-	-	28.72	YES
28	-	N/A	-	-	28.85	YES	-	-	28.81	YES
29	200x LOD	33,914	29.27	+	28.76	YES	28.62	+	28.96	YES
30	-	N/A	-	-	29.07	YES	-	-	28.90	YES
31	2.25x LOD	382	36.95	+	29.13	YES	34.76	+	29.15	YES
32	-	N/A	-	-	29.02	YES	-	-	28.75	YES
33	-	N/A	-	-	29.98	YES	-	-	29.83	YES
34	200x LOD	33,914	29.54	+	29.20	YES	28.91	+	29.44	YES
35	-	N/A	-	-	31.27	YES	-	-	31.22	YES
36	2.25x LOD	382	36.59	+	29.21	YES	34.17	+	29.19	YES
37	-	N/A	-	-	29.01	YES	-	-	28.82	YES
38	200x LOD	33,914	29.72	+	29.33	YES	28.81	+	29.27	YES
39	3x LOD	509	35.39	+	28.99	YES	34.21	+	28.85	YES
40	3x LOD	509	35.98	+	28.95	YES	33.99	+	28.83	YES

Table 17: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
41	-	N/A	-	-	28.50	YES	-	-	28.74	YES
42	2.25x LOD	382	35.22	+	28.68	YES	34.53	+	28.80	YES
43	-	N/A	-	-	29.09	YES	-	-	28.84	YES
44	-	N/A	-	-	28.95	YES	-	-	28.59	YES
45	200x LOD	33,914	29.21	+	28.67	YES	28.17	+	28.55	YES
46	-	N/A	-	-	29.09	YES	-	-	28.95	YES
47	-	N/A	-	-	28.92	YES	-	-	28.76	YES
48	2.25x LOD	382	36.72	+	29.19	YES	34.51	+	28.93	YES
49	2.25x LOD	382	36.59	+	28.99	YES	34.68	+	28.90	YES
50	-	N/A	-	-	29.03	YES	-	-	28.81	YES
51	3x LOD	509	36.12	+	28.72	YES	34.11	+	28.74	YES
52	3x LOD	509	35.22	+	28.94	YES	34.48	+	28.65	YES
53	200x LOD	33,914	29.31	+	28.86	YES	28.48	+	28.91	YES
54	-	N/A	-	-	28.78	YES	-	-	28.66	YES
55	-	N/A	-	-	29.74	YES	-	-	29.31	YES
56	200x LOD	33,914	29.40	+	28.99	YES	28.31	+	28.60	YES
57	-	N/A	-	-	28.97	YES	-	-	29.01	YES
58	-	N/A	-	-	29.07	YES	-	-	29.13	YES
59	3x LOD	509	35.44	+	29.03	YES	34.37	+	28.85	YES
60	-	N/A	-	-	28.78	YES	-	-	28.52	YES

Table 17: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
61	-	N/A	-	-	28.17	YES	-	-	28.07	YES
62	3x LOD	509	34.22	+	27.96	YES	33.59	+	27.76	YES
63	2.25x LOD	382	36.67	+	28.02	YES	34.22	+	27.76	YES
64	-	N/A	-	-	28.20	YES	-	-	27.99	YES
65	3x LOD	509	34.88	+	27.70	YES	34.14	+	27.60	YES
66	-	N/A	-	-	27.76	YES	-	-	27.69	YES
67	3x LOD	509	34.71	+	28.02	YES	33.93	+	27.73	YES
68	2.25x LOD	382	36.59	+	28.04	YES	34.32	+	27.87	YES
69	2.25x LOD	382	36.34	+	27.83	YES	34.80	+	27.94	YES
70	200x LOD	33,914	28.94	+	27.72	YES	28.07	+	27.71	YES
71	-	N/A	-	-	27.86	YES		-	27.51	YES
72	200x LOD	33,914	29.06	+	27.96	YES	28.06	+	27.67	YES
73	-	N/A	-	-	28.05	YES	-	-	27.73	YES
74	-	N/A	-	-	27.88	YES	-	-	27.71	YES
75	-	N/A	-	-	27.96	YES	-	-	27.84	YES
76	-	N/A	-	-	27.83	YES	-	-	27.63	YES
77	-	N/A	-	-	28.18	YES	-	-	28.01	YES
78	-	N/A	-	-	27.86	YES	-	-	27.63	YES
79	-	N/A	-	-	27.87	YES	-	-	27.71	YES
80	200x LOD	33,914	28.82	+	27.83	YES	28.03	+	27.55	YES

Table 17: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
81	-	N/A	-	-	27.89	YES	-	-	27.83	YES
82	-	N/A	-	-	27.81	YES	-	-	27.54	YES
83	200x LOD	33,914	28.76	+	27.46	YES	28.00	+	27.55	YES
84	-	N/A	-	-	27.82	YES	-	-	27.73	YES
85	-	N/A	-	-	28.02	YES	-	-	27.81	YES
86	200x LOD	33,914	29.06	+	28.11	YES	28.07	+	28.02	YES
87	2.25x LOD	382	37.31	+	27.89	YES	34.44	+	27.75	YES
88	2.25x LOD	382	35.97	+	27.93	YES	34.32	+	27.79	YES
89	-	N/A	-	-	28.01	YES	-	-	27.86	YES
90	3x LOD	509	35.11	+	28.00	YES	33.52	+	27.92	YES
91	-	N/A	-	-	27.85	YES	-	-	27.77	YES
92	-	N/A	-	-	27.94	YES	-	-	27.59	YES
93	2.25x LOD	382	34.49	+	27.76	YES	33.78	+	27.58	YES
94	-	N/A	-	-	27.98	YES	-	-	27.87	YES
95	200x LOD	33,914	30.04	+	29.08	YES	29.16	+	28.69	YES
96	-	N/A	-	-	29.14	YES	-	-	29.05	YES
97	3x LOD	509	36.40	+	28.00	YES	34.14	+	27.80	YES
98	-	N/A	-	-	27.89	YES	-	-	27.84	YES
99	-	N/A	-	-	27.59	YES	-	-	27.45	YES
100	3x LOD	509	36.30	+	28.30	YES	34.87	+	28.30	YES

Table 18: Mock Clinical Study on CFX96™/Dx Real-Time System - Summary Statistics

RealStar® MERS-CoV RT-PCR Kit U.S. in combination with the CFX96™ /Dx Real-Time System	*upE*			*orf1a*		
	Positive results		Negative results	Positive results		Negative results
2.25x LOD (15 samples)	15		0	15		0
3x LOD (15 samples)	15		0	15		0
200x LOD (15 samples)	15		0	15		0
Neg. specimens (55 samples)	0		55	0		55
Total (100 samples)	45		55	45		55
			95% CI			95% CI
Positive Percent Agreement	45/45	100%	92.1% - 100%	45/45	100%	92.1% - 100%
Negative Percent Agreement	55/55	100%	93.5% - 100%	55/55	100%	93.5% - 100%

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™/Dx Real-Time System correctly identified 45 of 45 specimens spiked with MERS-CoV RNA at the concentrations shown, including concentrations near the limit of detection of the assay. No unspiked specimen rendered a positive signal.

14.1.2 RealStar® MERS-CoV RT-PCR Kit U.S. on LightCycler® 480 Instrument II

The results of the analysis with the RealStar® MERS-CoV RT-PCR Kit U.S. on the LightCycler® 480 Instrument II (Roche) can be found in the tables below.

Table 19: Mock clinical study: RealStar® MERS-CoV RT-PCR Kit U.S. on LightCycler® 480 Instrument II

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
1	-	N/A	-	-	28.86	YES	-	-	28.72	YES
2	-	N/A	-	-	28.67	YES	-	-	28.58	YES
3	200x LOD	33,914	28.89	+	28.27	YES	28.34	+	27.99	YES
4	-	N/A	-	-	28.75	YES	-	-	28.77	YES
5	-	N/A	-	-	28.64	YES	-	-	28.59	YES
6	-	N/A	-	-	28.68	YES	-	-	28.7	YES
7	-	N/A	-	-	28.68	YES	-	-	28.54	YES
8	3x LOD	509	33.92	+	28.63	YES	34.9	+	28.56	YES
9	2.25x LOD	382	34.6	+	28.63	YES	35.07	+	28.58	YES
10	3x LOD	509	34.3	+	28.66	YES	34.5	+	28.6	YES
11	3x LOD	509	34.54	+	28.89	YES	34.25	+	28.77	YES
12	-	N/A	-	-	28.5	YES	-	-	28.47	YES
13	200x LOD	33,914	29.15	+	28.55	YES	28.53	+	28.14	YES
14	-	N/A	-	-	28.62	YES	-	-	28.52	YES
15	-	N/A	-	-	28.68	YES	-	-	28.56	YES
16	200x LOD	33,914	28.95	+	28.27	YES	28.24	+	27.97	YES
17	-	N/A	-	-	28.59	YES	-	-	28.62	YES
18	2.25x LOD	382	34.75	+	28.64	YES	35.29	+	28.65	YES
19	2.25x LOD	382	34.98	+	28.61	YES	34.72	+	28.58	YES
20	-	N/A	-	-	28.82	YES	-	-	28.73	YES

Table 19: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
21	-	N/A	-	-	28.16	YES	-	-	28.45	YES
22	-	N/A	-	-	27.94	YES	-	-	28.5	YES
23	-	N/A	-	-	27.63	YES	-	-	28.19	YES
24	2.25x LOD	382	34.06	+	28.16	YES	34.91	+	28.18	YES
25	-	N/A	-	-	28.05	YES	-	-	28.42	YES
26	3x LOD	509	34.04	+	28.04	YES	34.13	+	28.34	YES
27	-	N/A	-	-	28.2	YES	-	-	28.23	YES
28	-	N/A	-	-	28.28	YES	-	-	28.27	YES
29	200x LOD	33,914	28.9	+	27.99	YES	28.22	+	27.79	YES
30	-	N/A	-	-	28.6	YES	-	-	28.49	YES
31	2.25x LOD	382	33.35	+	28.24	YES	34.15	+	28.47	YES
32	-	N/A	-	-	28.03	YES	-	-	28.3	YES
33	-	N/A	-	-	29.11	YES	-	-	29.28	YES
34	200x LOD	33,914	28.71	+	27.77	YES	28.3	+	27.88	YES
35	-	N/A	-	-	29.85	YES	-	-	30.19	YES
36	2.25x LOD	382	33.71	+	28.25	YES	34.82	+	28.53	YES
37	-	N/A	-	-	28.16	YES	-	-	28.45	YES
38	200x LOD	33,914	29.24	+	28.58	YES	28.69	+	28.07	YES
39	3x LOD	509	33.73	+	28.22	YES	33.9	+	28.34	YES
40	3x LOD	509	34.15	+	28.29	YES	34.09	+	28.45	YES

Table 19: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
41	-	N/A	-	-	28.04	YES	-	-	28.45	YES
42	2.25x LOD	382	34.46	+	28.19	YES	34.18	+	28.47	YES
43	-	N/A	-	-	28.42	YES	-	-	28.34	YES
44	-	N/A	-	-	28.45	YES	-	-	28.3	YES
45	200x LOD	33,914	28.58	+	27.85	YES	27.95	+	27.58	YES
46	-	N/A	-	-	28.6	YES	-	-	28.47	YES
47	-	N/A	-	-	28.26	YES	-	-	28.13	YES
48	2.25x LOD	382	34.86	+	28.46	YES	34.13	+	28.63	YES
49	2.25x LOD	382	34.63	+	28.52	YES	34.18	+	28.57	YES
50	-	N/A	-	-	28.26	YES	-	-	28.45	YES
51	3x LOD	509	33.67	+	28.33	YES	34.5	+	28.45	YES
52	3x LOD	509	34.18	+	28.42	YES	33.65	+	28.36	YES
53	200x LOD	33,914	28.81	+	28.04	YES	28.32	+	27.9	YES
54	-	N/A	-	-	28.33	YES	-	-	-	INVALID
55	-	N/A	-	-	29.07	YES	-	-	29.15	YES
56	200x LOD	33,914	28.84	+	28.17	YES	28.32	+	27.93	YES
57	-	N/A	-	-	28.5	YES	-	-	28.44	YES
58	-	N/A	-	-	28.29	YES	-	-	28.55	YES
59	3x LOD	509	34.21	+	28.52	YES	34.57	+	28.45	YES
60	-	N/A	-	-	28.43	YES	-	-	28.29	YES

Table 19: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
61	-	N/A	-	-	26.87	YES	-	-	28.46	YES
62	3x LOD	509	34.01	+	27.93	YES	33.69	+	28.16	YES
63	2.25x LOD	382	34.29	+	28.01	YES	34.99	+	28.24	YES
64	-	N/A	-	-	28.66	YES	-	-	28.71	YES
65	3x LOD	509	33.75	+	27.99	YES	33.96	+	28.29	YES
66	-	N/A	-	-	27.77	YES	-	-	28.1	YES
67	3x LOD	509	33.55	+	28.19	YES	34.17	+	28.48	YES
68	2.25x LOD	382	33.17	+	27.83	YES	34.35	+	28.3	YES
69	2.25x LOD	382	34.01	+	28.26	YES	34.82	+	28.24	YES
70	200x LOD	33,914	28.88	+	27.91	YES	28.24	+	27.74	YES
71	-	N/A	-	-	28.12	YES	-	-	28.43	YES
72	200x LOD	33,914	28.73	+	27.7	YES	28.25	+	27.77	YES
73	-	N/A	-	-	27.94	YES	-	-	28.15	YES
74	-	N/A	-	-	28.2	YES	-	-	28.21	YES
75	-	N/A	-	-	27.24	YES	-	-	28.16	YES
76	-	N/A	-	-	28.21	YES	-	-	28.34	YES
77	-	N/A	-	-	28.46	YES	-	-	28.5	YES
78	-	N/A	-	-	27.98	YES	-	-	28.2	YES
79	-	N/A	-	-	27.5	YES	-	-	28.17	YES
80	200x LOD	33,914	28.87	+	28.02	YES	28.3	+	27.88	YES

Table 19: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
81	-	N/A	-	-	27.2	YES	-	-	28.12	YES
82	-	N/A	-	-	28.25	YES	-	-	28.35	YES
83	200x LOD	33,914	28.73	+	27.82	YES	28.11	+	27.67	YES
84	-	N/A	-	-	28.12	YES	-	-	28.33	YES
85	-	N/A	-	-	28.19	YES	-	-	28.48	YES
86	200x LOD	33,914	28.98	+	28.1	YES	28.27	+	27.89	YES
87	2.25x LOD	382	34.75	+	28.23	YES	34.64	+	28.34	YES
88	2.25x LOD	382	34.33	+	28.1	YES	34.1	+	28.33	YES
89	-	N/A	-	-	28.47	YES	-	-	28.5	YES
90	3x LOD	509	33.78	+	28.3	YES	34.82	+	28.66	YES
91	-	N/A	-	-	28.13	YES	-	-	28.44	YES
92	-	N/A	-	-	28.27	YES	-	-	28.18	YES
93	2.25x LOD	382	33.32	+	27.99	YES	33.75	+	28.26	YES
94	-	N/A	-	-	28.26	YES	-	-	28.26	YES
95	200x LOD	33,914	29.97	+	29.13	YES	29.66	+	29.02	YES
96	-	N/A	-	-	29.23	YES	-	-	29.58	YES
97	3x LOD	509	32.19	+	27.9	YES	34.13	+	28.35	YES
98	-	N/A	-	-	28.26	YES	-	-	28.29	YES
99	-	N/A	-	-	28.34	YES	-	-	28.14	YES
100	3x LOD	509	35.44	+	28.94	YES	34.87	+	28.95	YES

Table 20: Mock Clinical Study on LightCycler® 480 Instrument II - Summary Statistics

RealStar® MERS-CoV RT-PCR Kit U.S. in combination with the LightCycler® 480 Instrument II	*upE*		*orf1a*		
	Positive results	Negative results	Positive results	Negative results	
2.25x LOD (15 samples)	15	0	15	0	
3x LOD (15 samples)	15	0	15	0	
200x LOD (15 samples)	15	0	15	0	
Neg. specimens (55 samples)	0	55	0	54*	
Total (100/99 samples)	45	55	45	54*	
		95% CI		95% CI	
Positive Percent Agreement	45/45	100%	45/45	100%	92.1% - 100%
Negative Percent Agreement	55/55	100%	54/54	100%	93.4% - 100%

* One MERS-CoV negative sample (no. 54) was excluded from the statistical analysis for the *orft1a* specific assay, since no signal could be observed in the JOE/VIC channel for the Internal Control making the result generated for this sample invalid.

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the LightCycler® 480 Instrument II correctly identified 45 of 45 specimens spiked with MERS-CoV RNA at the concentrations shown, including concentrations near the limit of detection of the assay. No unspiked specimen rendered a positive signal.

14.1.3 RealStar® MERS-CoV RT-PCR Kit U.S. on the Rotor-Gene® 6000

The results of the analysis with the RealStar® MERS-CoV RT-PCR Kit U.S. on Rotor-Gene® 6000 (Corbett Research) can be found in the tables below.

Table 21: Mock clinical study: RealStar® MERS-CoV RT-PCR Kit U.S. on Rotor-Gene® 6000

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
1	-	N/A	-	-	28.02	YES	-	-	27.75	YES
2	-	N/A	-	-	27.88	YES	-	-	27.57	YES
3	200x LOD	33,914	28.52	+	27.77	YES	26.69	+	27.22	YES
4	-	N/A	-	-	28.13	YES	-	-	27.87	YES
5	-	N/A	-	-	27.87	YES	-	-	27.59	YES
6	-	N/A	-	-	27.89	YES	-	-	27.64	YES
7	-	N/A	-	-	27.81	YES	-	-	27.7	YES
8	3x LOD	509	35.5	+	27.84	YES	33.15	+	27.42	YES
9	2.25x LOD	382	38.35	+	27.82	YES	33.08	+	27.46	YES
10	3x LOD	509	35.17	+	27.98	YES	32.75	+	27.5	YES
11	3x LOD	509	34.9	+	28.18	YES	32.26	+	27.91	YES
12	-	N/A	-	-	27.56	YES	-	-	27.44	YES
13	200x LOD	33,914	28.65	+	27.76	YES	26.99	+	27.51	YES
14	-	N/A	-	-	27.62	YES	-	-	27.53	YES
15	-	N/A	-	-	27.81	YES	-	-	27.66	YES
16	200x LOD	33,914	28.56	+	27.69	YES	26.74	+	27.42	YES
17	-	N/A	-	-	27.81	YES	-	-	27.7	YES
18	2.25x LOD	382	37.57	+	27.72	YES	32.76	+	27.64	YES
19	2.25x LOD	382	35.69	+	27.64	YES	33.17	+	27.52	YES
20	-	N/A	-	-	28.22	YES	-	-	28.07	YES

Table 21: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
21	-	N/A	-	-	27.26	YES	-	-	27.42	YES
22	-	N/A	-	-	27.66	YES	-	-	27.64	YES
23	-	N/A	-	-	27.41	YES	-	-	27.46	YES
24	2.25x LOD	382	35.74	+	27.44	YES	32.98	+	27.28	YES
25	-	N/A	-	-	27.49	YES	-	-	27.52	YES
26	3x LOD	509	35.04	+	27.5	YES	32.71	+	27.29	YES
27	-	N/A	-	-	27.42	YES	-	-	27.21	YES
28	-	N/A	-	-	27.5	YES	-	-	27.23	YES
29	200x LOD	33,914	28.77	+	27.46	YES	26.83	+	27.07	YES
30	-	N/A	-	-	27.54	YES	-	-	27.31	YES
31	2.25x LOD	382	37.99	+	27.53	YES	32.98	+	27.31	YES
32	-	N/A	-	-	27.34	YES	-	-	27.15	YES
33	-	N/A	-	-	28.54	YES	-	-	28.17	YES
34	200x LOD	33,914	28.59	+	27.66	YES	27.09	+	27.51	YES
35	-	N/A	-	-	28.87	YES	-	-	28.78	YES
36	2.25x LOD	382	37.87	+	27.98	YES	34.22	+	27.85	YES
37	-	N/A	-	-	27.73	YES	-	-	27.24	YES
38	200x LOD	33,914	28.75	+	27.67	YES	27.27	+	27.66	YES
39	3x LOD	509	35.27	+	27.39	YES	32.59	+	27.18	YES
40	3x LOD	509	35.69	+	27.39	YES	32.42	+	27.04	YES

Table 21: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
41	-	N/A	-	-	27.89	YES	-	-	27.45	YES
42	2.25x LOD	382	35.93	+	27.75	YES	33.78	+	27.58	YES
43	-	N/A	-	-	27.92	YES	-	-	27.71	YES
44	-	N/A	-	-	27.71	YES	-	-	27.29	YES
45	200x LOD	33,914	28.35	+	27.44	YES	26.54	+	27.08	YES
46	-	N/A	-	-	27.59	YES	-	-	27.64	YES
47	-	N/A	-	-	27.68	YES	-	-	27.02	YES
48	2.25x LOD	382	36.26	+	27.88	YES	33.11	+	27.55	YES
49	2.25x LOD	382	35.69	+	27.81	YES	32.35	+	27.26	YES
50	-	N/A	-	-	27.84	YES	-	-	27.49	YES
51	3x LOD	509	35.55	+	27.77	YES	33.03	+	27.58	YES
52	3x LOD	509	35.27	+	27.54	YES	32.98	+	27.35	YES
53	200x LOD	33,914	28.57	+	27.52	YES	26.68	+	27.12	YES
54	-	N/A	-	-	27.51	YES	-	-	27.31	YES
55	-	N/A	-	-	28.36	YES	-	-	28.1	YES
56	200x LOD	33,914	28.42	+	27.49	YES	26.49	+	27.14	YES
57	-	N/A	-	-	27.85	YES	-	-	27.52	YES
58	-	N/A		-	27.59	YES		-	27.49	YES
59	3x LOD	509	35.38	+	27.78	YES	32.56	+	27.53	YES
60	-	N/A	-	-	27.45	YES	-	-	27.32	YES

Table 21: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
61	-	N/A	-	-	28.09	YES	-	-	27.83	YES
62	3x LOD	509	35.19	+	27.76	YES	31.8	+	27.51	YES
63	2.25x LOD	382	36.13	+	27.76	YES	32.88	+	27.77	YES
64	-	N/A	-	-	28.2	YES	-	-	27.84	YES
65	3x LOD	509	34.75	+	27.74	YES	33.04	+	27.48	YES
66	-	N/A	-	-	27.88	YES	-	-	27.47	YES
67	3x LOD	509	35.33	+	27.98	YES	32.47	+	27.51	YES
68	2.25x LOD	382	35.81	+	27.63	YES	32.83	+	27.64	YES
69	2.25x LOD	382	36.9	+	27.77	YES	33.3	+	27.76	YES
70	200x LOD	33,914	28.39	+	27.26	YES	26.77	+	27.36	YES
71	-	N/A	-	-	27.8	YES	-	-	27.66	YES
72	200x LOD	33,914	28.56	+	27.62	YES	26.89	+	27.5	YES
73	-	N/A	-	-	27.8	YES	-	-	27.67	YES
74	-	N/A	-	-	27.72	YES	-	-	27.55	YES
75	-	N/A	-	-	27.71	YES	-	-	27.62	YES
76	-	N/A	-	-	27.97	YES	-	-	27.41	YES
77	-	N/A	-	-	27.94	YES	-	-	27.49	YES
78	-	N/A	-	-	27.74	YES	-	-	27.29	YES
79	-	N/A	-	-	27.75	YES	-	-	27.47	YES
80	200x LOD	33,914	28.51	+	27.71	YES	26.75	+	27.4	YES

Table 21: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
81	-	N/A	-	-	27.62	YES	-	-	27.47	YES
82	-	N/A	-	-	27.76	YES	-	-	27.64	YES
83	200x LOD	33,914	28.41	+	27.32	YES	26.85	+	27.39	YES
84	-	N/A	-	-	27.86	YES	-	-	27.88	YES
85	-	N/A	-	-	27.73	YES	-	-	27.78	YES
86	200x LOD	33,914	28.55	+	27.76	YES	26.81	+	27.47	YES
87	2.25x LOD	382	37.07	+	27.83	YES	33.2	+	27.48	YES
88	2.25x LOD	382	35.14	+	27.71	YES	32.8	+	27.55	YES
89	-	N/A	-	-	27.71	YES	-	-	27.64	YES
90	3x LOD	509	35.41	+	28.05	YES	32.4	+	27.59	YES
91	-	N/A	-	-	27.69	YES	-	-	27.75	YES
92	-	N/A	-	-	27.65	YES	-	-	27.4	YES
93	2.25x LOD	382	35.33	+	27.79	YES	32.74	+	27.34	YES
94	-	N/A	-	-	27.69	YES	-	-	27.64	YES
95	200x LOD	33,914	29.84	+	29.4	YES	28.02	+	28.84	YES
96	-	N/A	-	-	29.12	YES	-	-	28.73	YES
97	3x LOD	509	35.73	+	27.71	YES	32.51	+	27.59	YES
98	-	N/A	-	-	27.76	YES	-	-	27.53	YES
99	-	N/A	-	-	27.81	YES	-	-	27.28	YES
100	3x LOD	509	36.66	+	28.59	YES	33.62	+	28.08	YES

Table 22: Mock Clinical Study on Rotor-Gene® 6000 - Summary Statistics

RealStar® MERS-CoV RT-PCR Kit U.S. in combination with the Rotor-Gene® 6000	*upE*			*orf1a*		
	Positive results		Negative results	Positive results		Negative results
2.25x LOD (15 samples)	15		0	15		0
3x LOD (15 samples)	15		0	15		0
200x LOD (15 samples)	15		0	15		0
Neg. specimens (55 samples)	0		55	0		55
Total (100 samples)	45		55	45		55
			95% CI			95% CI
Positive Percent Agreement	45/45	100%	92.1% - 100%	45/45	100%	92.1% - 100%
Negative Percent Agreement	55/55	100%	93.5% - 100%	55/55	100%	93.5% - 100%

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the Rotor-Gene® 6000 correctly identified 45 of 45 specimens spiked with MERS-CoV RNA at the concentrations shown, including concentrations near the limit of detection of the assay. No unspiked specimen rendered a positive signal.

14.1.4 RealStar® MERS-CoV RT-PCR Kit U.S. on the ABI Prism® 7500 SDS

The results of the analysis with the RealStar® MERS-CoV RT-PCR Kit U.S. on the ABI Prism® 7500 SDS (Applied Biosystems) can be found in the tables below.

Table 23: Mock clinical study: RealStar® MERS-CoV RT-PCR Kit U.S. on ABI Prism® 7500 SDS

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
1	-	N/A	-	-	29.37	YES	-	-	29.44	YES
2	-	N/A	-	-	29.50	YES	-	-	29.20	YES
3	200x LOD	33,914	29.39	+	29.38	YES	28.37	+	28.92	YES
4	-	N/A	-	-	29.56	YES	-	-	29.43	YES
5	-	N/A	-	-	29.76	YES	-	-	29.35	YES
6	-	N/A	-	-	29.57	YES	-	-	29.22	YES
7	-	N/A	-	-	29.54	YES	-	-	29.27	YES
8	3x LOD	509	35.38	+	29.38	YES	34.62	+	29.45	YES
9	2.25x LOD	382	35.75	+	29.56	YES	34.29	+	28.57	YES
10	3x LOD	509	36.62	+	29.36	YES	34.50	+	29.23	YES
11	3x LOD	509	36.18	+	29.77	YES	34.27	+	29.43	YES
12	-	N/A	-	-	29.31	YES	-	-	29.16	YES
13	200x LOD	33,914	29.70	+	29.59	YES	28.86	+	29.30	YES
14	-	N/A	-	-	29.36	YES	-	-	29.13	YES
15	-	N/A	-	-	29.39	YES	-	-	29.19	YES
16	200x LOD	33,914	28.39	+	28.25	YES	28.61	+	29.22	YES
17	-	N/A	-	-	29.59	YES	-	-	29.08	YES
18	2.25x LOD	382	36.68	+	29.55	YES	34.20	+	29.26	YES
19	2.25x LOD	382	36.13	+	29.34	YES	34.51	+	29.18	YES
20	-	N/A	-	-	29.81	YES	-	-	29.49	YES

Table 23: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
21	-	N/A	-	-	29.10	YES	-	-	28.96	YES
22	-	N/A	-	-	29.12	YES	-	-	29.14	YES
23	-	N/A	-	-	28.96	YES	-	-	28.88	YES
24	2.25x LOD	382	35.69	+	29.04	YES	35.15	+	28.92	YES
25	-	N/A	-	-	29.15	YES	-	-	29.26	YES
26	3x LOD	509	36.34	+	29.27	YES	34.26	+	29.04	YES
27	-	N/A	-	-	28.99	YES	-	-	28.97	YES
28	-	N/A	-	-	28.94	YES	-	-	28.86	YES
29	200x LOD	33,914	29.41	+	29.21	YES	28.62	+	29.00	YES
30	-	N/A	-	-	27.02	YES	-	-	29.20	YES
31	2.25x LOD	382	40.23	+	29.06	YES	34.28	+	29.05	YES
32	-	N/A	-	-	29.13	YES	-	-	28.80	YES
33	-	N/A	-	-	30.02	YES	-	-	29.96	YES
34	200x LOD	33,914	29.28	+	29.43	YES	28.60	+	29.01	YES
35	-	N/A	-	-	30.96	YES	-	-	31.12	YES
36	2.25x LOD	382	38.08	+	29.32	YES	34.63	+	29.18	YES
37	-	N/A	-	-	29.26	YES	-	-	28.95	YES
38	200x LOD	33,914	29.82	+	29.93	YES	28.96	+	29.55	YES
39	3x LOD	509	36.23	+	29.16	YES	33.84	+	28.86	YES
40	3x LOD	509	36.62	+	29.10	YES	34.09	+	28.98	YES

Table 23: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
41	-	N/A	-	-	29.18	YES	-	-	28.61	YES
42	2.25x LOD	382	37.31	+	29.30	YES	34.25	+	29.07	YES
43	-	N/A	-	-	29.23	YES	-	-	29.29	YES
44	-	N/A	-	-	29.02	YES	-	-	29.02	YES
45	200x LOD	33,914	29.20	+	28.92	YES	28.17	+	28.65	YES
46	-	N/A	-	-	29.28	YES	-	-	28.98	YES
47	-	N/A	-	-	29.58	YES	-	-	28.96	YES
48	2.25x LOD	382	38.25	+	29.42	YES	35.55	+	29.19	YES
49	2.25x LOD	382	35.74	+	29.12	YES	34.26	+	29.34	YES
50	-	N/A	-	-	29.24	YES	-	-	29.03	YES
51	3x LOD	509	35.78	+	29.22	YES	35.42	+	29.22	YES
52	3x LOD	509	35.93	+	29.16	YES	34.69	+	28.93	YES
53	200x LOD	33,914	29.29	+	29.14	YES	28.46	+	29.06	YES
54	-	N/A	-	-	29.11	YES	-	-	28.95	YES
55	-	N/A	-	-	32.94	YES	-	-	29.89	YES
56	200x LOD	33,914	29.22	+	29.08	YES	28.33	+	28.90	YES
57	-	N/A	-	-	29.26	YES	-	-	29.18	YES
58	-	N/A	-	-	29.40	YES	-	-	29.14	YES
59	3x LOD	509	35.78	+	29.39	YES	34.08	+	28.97	YES
60	-	N/A	-	-	29.10	YES	-	-	29.01	YES

Table 23: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
61	-	N/A	-	-	29.08	YES	-	-	29.29	YES
62	3x LOD	509	35.35	+	28.95	YES	33.82	+	29.12	YES
63	2.25x LOD	382	39.13	+	29.57	YES	34.86	+	29.24	YES
64	-	N/A	-	-	29.53	YES	-	-	29.44	YES
65	3x LOD	509	37.30	+	29.22	YES	34.56	+	28.98	YES
66	-	N/A	-	-	28.94	YES	-	-	28.94	YES
67	3x LOD	509	38.22	+	29.95	YES	34.42	+	29.19	YES
68	2.25x LOD	382	37.97	+	29.03	YES	34.60	+	28.96	YES
69	2.25x LOD	382	37.99	+	29.21	YES	34.57	+	29.16	YES
70	200x LOD	33,914	29.41	+	29.47	YES	28.70	+	29.10	YES
71	-	N/A	-	-	29.18	YES	-	-	29.04	YES
72	200x LOD	33,914	29.00	+	28.96	YES	28.56	+	29.08	YES
73	-	N/A	-	-	29.12	YES	-	-	28.82	YES
74	-	N/A	-	-	29.13	YES	-	-	28.86	YES
75	-	N/A	-	-	28.92	YES	-	-	28.95	YES
76	-	N/A	-	-	29.24	YES	-	-	28.86	YES
77	-	N/A	-	-	29.40	YES	-	-	29.15	YES
78	-	N/A	-	-	29.18	YES	-	-	29.17	YES
79	-	N/A	-	-	29.18	YES	-	-	28.85	YES
80	200x LOD	33,914	29.15	+	29.21	YES	28.39	+	28.85	YES

Table 23: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
81	-	N/A	-	-	28.98	YES	-	-	29.04	YES
82	-	N/A	-	-	29.10	YES	-	-	28.98	YES
83	200x LOD	33,914	29.21	+	28.97	YES	28.35	+	28.93	YES
84	-	N/A	-	-	29.10	YES	-	-	28.97	YES
85	-	N/A	-	-	29.28	YES	-	-	29.18	YES
86	200x LOD	33,914	29.27	+	29.26	YES	28.28	+	28.91	YES
87	2.25x LOD	382	35.48	+	29.25	YES	35.24	+	29.20	YES
88	2.25x LOD	382	36.74	+	29.09	YES	34.38	+	28.95	YES
89	-	N/A	-	-	29.20	YES	-	-	29.13	YES
90	3x LOD	509	34.81	+	29.34	YES	34.17	+	29.38	YES
91	-	N/A	-	-	28.90	YES	-	-	29.29	YES
92	-	N/A	-	-	29.15	YES	-	-	28.93	YES
93	2.25x LOD	382	35.90	+	29.05	YES	34.26	+	28.96	YES
94	-	N/A	-	-	29.16	YES	-	-	28.98	YES
95	200x LOD	33,914	30.48	+	30.36	YES	29.95	+	30.75	YES
96	-	N/A	-	-	30.21	YES	-	-	30.04	YES
97	3x LOD	509	37.97	+	29.09	YES	34.47	+	29.26	YES
98	-	N/A	-	-	29.40	YES	-	-	28.94	YES
99	-	N/A	-	-	29.27	YES	-	-	28.91	YES
100	3x LOD	509	37.43	+	29.90	YES	35.30	+	29.84	YES

Table 24: Mock Clinical Study on ABI Prism® 7500 SDS - Summary Statistics

RealStar® MERS-CoV RT-PCR Kit U.S. in combination with the ABI Prism® 7500 SDS	*upE*			*orf1a*		
	Positive results		Negative results	Positive results		Negative results
2.25x LOD (15 samples)	15		0	15		0
3x LOD (15 samples)	15		0	15		0
200x LOD (15 samples)	15		0	15		0
Neg. specimens (55 samples)	0		55	0		55
Total (100 samples)	45		55	45		55
			95% CI			95% CI
Positive Percent Agreement	45/45	100%	92.1% - 100%	45/45	100%	92.1% - 100%
Negative Percent Agreement	55/55	100%	93.5% - 100%	55/55	100%	93.5% - 100%

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the ABI Prism® 7500 SDS correctly identified 45 of 45 specimens spiked with MERS-CoV RNA at the concentrations shown, including concentrations near the limit of detection of the assay. No unspiked specimen rendered a positive signal.

14.1.5 RealStar® MERS-CoV RT-PCR Kit U.S. on VERSANT® kPCR Molecular System AD

The results of the analysis with the RealStar® MERS-CoV RT-PCR Kit U.S. on the VERSANT® kPCR Molecular System AD (Siemens) can be found in the tables below.

Table 25: Mock clinical study: RealStar® MERS-CoV RT-PCR Kit U.S. on VERSANT® kPCR Molecular System AD

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agree-ment w/ expected result	Cp value	Call	IC Cp	Agree-ment w/ expected result
1	-	N/A	-	-	29.57	YES	-	-	29.70	YES
2	-	N/A	-	-	29.23	YES	-	-	29.11	YES
3	200x LOD	33,914	29.78	+	28.99	YES	28.94	+	28.51	YES
4	-	N/A	-	-	29.46	YES	-	-	29.18	YES
5	-	N/A	-	-	28.83	YES	-	-	28.89	YES
6	-	N/A	-	-	29.35	YES	-	-	29.15	YES
7	-	N/A	-	-	28.93	YES	-	-	29.80	YES
8	3x LOD	509	35.43	+	29.31	YES	34.47	+	28.80	YES
9	2.25x LOD	382	35.72	+	29.80	YES	35.12	+	29.70	YES
10	3x LOD	509	35.62	+	28.85	YES	33.99	+	28.99	YES
11	3x LOD	509	36.55	+	29.25	YES	33.98	+	29.27	YES
12	-	N/A	-	-	29.11	YES	-	-	28.51	YES
13	200x LOD	33,914	30.11	+	29.41	YES	29.13	+	28.70	YES
14	-	N/A	-	-	28.96	YES	-	-	28.69	YES
15	-	N/A	-	-	29.37	YES	-	-	28.79	YES
16	200x LOD	33,914	29.95	+	29.11	YES	28.96	+	28.63	YES
17	-	N/A	-	-	29.25	YES	-	-	29.40	YES
18	2.25x LOD	382	35.90	+	29.26	YES	34.64	+	29.50	YES
19	2.25x LOD	382	36.76	+	29.25	YES	34.69	+	28.73	YES
20	-	N/A	-	-	29.36	YES	-	-	28.93	YES

Table 25: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
21	-	N/A	-	-	28.95	YES	-	-	28.46	YES
22	-	N/A	-	-	28.41	YES	-	-	28.30	YES
23	-	N/A	-	-	28.37	YES	-	-	28.44	YES
24	2.25x LOD	382	37.85	+	28.53	YES	34.44	+	28.14	YES
25	-	N/A	-	-	28.69	YES	-	-	28.41	YES
26	3x LOD	509	36.83	+	28.67	YES	34.53	+	28.56	YES
27	-	N/A	-	-	28.81	YES	-	-	28.38	YES
28	-	N/A	-	-	28.74	YES	-	-	28.70	YES
29	200x LOD	33,914	29.72	+	28.67	YES	28.63	+	28.23	YES
30	-	N/A	-	-	28.70	YES	-	-	29.00	YES
31	2.25x LOD	382	37.08	+	28.89	YES	34.59	+	28.41	YES
32	-	N/A	-	-	28.22	YES	-	-	28.14	YES
33	-	N/A	-	-	29.52	YES	-	-	29.58	YES
34	200x LOD	33,914	29.36	+	28.48	YES	28.94	+	28.71	YES
35	-	N/A	-	-	30.32	YES	-	-	30.49	YES
36	2.25x LOD	382	37.71	+	29.21	YES	35.79	+	29.11	YES
37	-	N/A	-	-	28.82	YES	-	-	28.38	YES
38	200x LOD	33,914	29.82	+	29.18	YES	29.40	+	29.90	YES
39	3x LOD	509	35.34	+	28.11	YES	34.84	+	28.18	YES
40	3x LOD	509	35.99	+	28.40	YES	34.51	+	28.11	YES

Table 25: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
41	-	N/A	-	-	28.77	YES	-	-	28.13	YES
42	2.25x LOD	382	35.91	+	28.70	YES	34.24	+	27.86	YES
43	-	N/A	-	-	28.58	YES	-	-	28.33	YES
44	-	N/A	-	-	28.53	YES	-	-	28.73	YES
45	200x LOD	33,914	29.36	+	28.58	YES	28.39	+	27.96	YES
46	-	N/A	-	-	28.99	YES	-	-	29.00	YES
47	-	N/A	-	-	28.69	YES	-	-	28.19	YES
48	2.25x LOD	382	36.19	+	29.16	YES	35.67	+	28.98	YES
49	2.25x LOD	382	35.23	+	28.83	YES	34.79	+	28.65	YES
50	-	N/A	-	-	28.46	YES	-	-	27.92	YES
51	3x LOD	509	35.64	+	28.71	YES	34.52	+	28.52	YES
52	3x LOD	509	35.93	+	28.78	YES	34.88	+	28.68	YES
53	200x LOD	33,914	29.61	+	28.64	YES	28.87	+	28.48	YES
54	-	N/A	-	-	28.58	YES	-	-	28.39	YES
55	-	N/A	-	-	29.61	YES	-	-	29.17	YES
56	200x LOD	33,914	29.97	+	28.93	YES	28.98	+	28.32	YES
57	-	N/A	-	-	28.86	YES	-	-	28.92	YES
58	-	N/A	-	-	28.65	YES	-	-	28.80	YES
59	3x LOD	509	35.04	+	28.39	YES	34.11	+	28.25	YES
60	-	N/A	-	-	28.88	YES	-	-	28.55	YES

Table 25: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
61	-	N/A	-	-	28.13	YES	-	-	28.68	YES
62	3x LOD	509	35.44	+	27.78	YES	34.58	+	27.70	YES
63	2.25x LOD	382	35.47	+	28.10	YES	35.50	+	28.69	YES
64	-	N/A	-	-	28.41	YES	-	-	28.21	YES
65	3x LOD	509	35.55	+	28.50	YES	34.35	+	27.92	YES
66	-	N/A	-	-	27.91	YES	-	-	28.19	YES
67	3x LOD	509	35.32	+	28.50	YES	34.81	+	28.19	YES
68	2.25x LOD	382	36.87	+	28.18	YES	34.77	+	28.21	YES
69	2.25x LOD	382	36.03	+	28.53	YES	35.01	+	28.63	YES
70	200x LOD	33,914	29.56	+	27.59	YES	28.68	+	27.43	YES
71	-	N/A	-	-	28.15	YES	-	-	28.12	YES
72	200x LOD	33,914	29.74	+	28.30	YES	29.02	+	28.27	YES
73	-	N/A	-	-	28.16	YES	-	-	28.16	YES
74	-	N/A	-	-	27.73	YES	-	-	28.63	YES
75	-	N/A	-	-	27.99	YES	-	-	28.44	YES
76	-	N/A	-	-	28.10	YES	-	-	28.11	YES
77	-	N/A	-	-	28.67	YES	-	-	28.31	YES
78	-	N/A	-	-	28.70	YES	-	-	27.97	YES
79	-	N/A	-	-	27.83	YES	-	-	27.90	YES
80	200x LOD	33,914	29.50	+	27.79	YES	28.79	+	27.81	YES

Table 25: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
81	-	N/A	-	-	27.80	YES	-	-	27.92	YES
82	-	N/A	-	-	28.14	YES	-	-	27.32	YES
83	200x LOD	33,914	29.46	+	27.65	YES	28.48	+	27.17	YES
84	-	N/A	-	-	27.93	YES	-	-	28.15	YES
85	-	N/A	-	-	28.37	YES	-	-	28.80	YES
86	200x LOD	33,914	29.31	+	27.87	YES	28.62	+	27.60	YES
87	2.25x LOD	382	35.85	+	28.26	YES	34.65	+	28.22	YES
88	2.25x LOD	382	36.50	+	28.90	YES	36.22	+	28.24	YES
89	-	N/A	-	-	28.63	YES	-	-	28.42	YES
90	3x LOD	509	35.49	+	27.96	YES	35.24	+	27.61	YES
91	-	N/A	-	-	28.14	YES	-	-	27.97	YES
92	-	N/A	-	-	28.11	YES	-	-	27.96	YES
93	2.25x LOD	382	35.84	+	28.24	YES	34.91	+	28.12	YES
94	-	N/A	-	-	27.89	YES	-	-	27.76	YES
95	200x LOD	33,914	31.18	+	29.40	YES	29.96	+	29.25	YES
96	-	N/A	-	-	29.84	YES	-	-	29.61	YES
97	3x LOD	509	38.22	+	28.42	YES	34.67	+	28.28	YES
98	-	N/A	-	-	28.14	YES	-	-	28.90	YES
99	-	N/A	-	-	27.72	YES	-	-	27.51	YES
100	3x LOD	509	37.12	+	29.39	YES	35.97	+	29.90	YES

Table 26: Mock Clinical Study on VERSANT® kPCR Molecular System AD - Summary Statistics

RealStar® MERS-CoV RT-PCR Kit U.S. in combination with the VERSANT® kPCR Molecular System AD	*upE*			*orf1a*		
	Positive results		Negative results	Positive results		Negative results
2.25x LOD (15 samples)	15		0	15		0
3x LOD (15 samples)	15		0	15		0
200x LOD (15 samples)	15		0	15		0
Neg. specimens (55 samples)	0		55	0		55
Total (100 samples)	45		55	45		55
			95% CI			95% CI
Positive Percent Agreement	45/45	100%	92.1% - 100%	45/45	100%	92.1% - 100%
Negative Percent Agreement	55/55	100%	93.5% - 100%	55/55	100%	93.5% - 100%

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the VERSANT® kPCR Molecular System AD correctly identified 45 of 45 specimens spiked with MERS-CoV RNA at the concentrations shown, including concentrations near the limit of detection of the assay. No unspiked specimen rendered a positive signal.

14.1.6 RealStar® MERS-CoV RT-PCR Kit U.S. on CFX96 Touch™ Deep Well Real-Time PCR Detection System

The results of the analysis with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96 Touch™ Deep Well Real-Time PCR Detection System (BIO-RAD) can be found in the tables below..

Table 27: Mock clinical study: RealStar® MERS-CoV RT-PCR Kit U.S. on CFX96 Touch™ Deep Well Real-Time PCR Detection System

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
1	-	N/A	-	-	29.39	YES	-	-	29.22	YES
2	-	N/A	-	-	29.48	YES	-	-	29.26	YES
3	200x LOD	33,914	29.75	+	28.73	YES	29.28	+	28.86	YES
4	-	N/A	-	-	29.07	YES	-	-	29.11	YES
5	-	N/A	-	-	29.18	YES	-	-	28.92	YES
6	-	N/A	-	-	29.21	YES	-	-	29.29	YES
7	-	N/A	-	-	29.19	YES	-	-	29.21	YES
8	3x LOD	509	36.24	+	29.08	YES	34.62	+	29.05	YES
9	2.25x LOD	382	37.58	+	29.33	YES	36.35	+	29.42	YES
10	3x LOD	509	36.63	+	29.30	YES	34.58	+	28.99	YES
11	3x LOD	509	35.15	+	28.47	YES	35.33	+	29.32	YES
12	-	N/A	-	-	28.85	YES	-	-	28.64	YES
13	200x LOD	33,914	30.36	+	29.40	YES	29.41	+	28.98	YES
14	-	N/A	-	-	28.80	YES	-	-	28.88	YES
15	-	N/A	-	-	29.36	YES	-	-	28.83	YES
16	200x LOD	33,914	30.39	+	29.24	YES	29.27	+	28.80	YES
17	-	N/A	-	-	29.22	YES	-	-	28.79	YES
18	2.25x LOD	382	36.42	+	29.14	YES	35.91	+	29.09	YES
19	2.25x LOD	382	36.58	+	29.15	YES	35.78	+	29.13	YES
20	-	N/A	-	-	29.34	YES	-	-	29.10	YES

Table 27: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
21	-	N/A	-	-	28.61	YES	-	-	28.50	YES
22	-	N/A	-	-	28.48	YES	-	-	27.93	YES
23	-	N/A	-	-	28.47	YES	-	-	28.07	YES
24	2.25x LOD	382	36.72	+	30.41	YES	34.27	+	28.02	YES
25	-	N/A	-	-	28.56	YES	-	-	28.28	YES
26	3x LOD	509	36.02	+	28.67	YES	34.13	+	28.39	YES
27	-	N/A	-	-	28.28	YES	-	-	28.18	YES
28	-	N/A	-	-	28.75	YES	-	-	28.16	YES
29	200x LOD	33,914	29.74	+	28.81	YES	28.59	+	28.32	YES
30	-	N/A	-	-	28.47	YES	-	-	28.36	YES
31	2.25x LOD	382	36.13	+	28.36	YES	34.28	+	27.92	YES
32	-	N/A	-	-	28.43	YES	-	-	28.05	YES
33	-	N/A	-	-	29.69	YES	-	-	29.04	YES
34	200x LOD	33,914	29.33	+	28.47	YES	28.53	+	28.34	YES
35	-	N/A	-	-	30.37	YES	-	-	30.36	YES
36	2.25x LOD	382	37.38	+	28.78	YES	35.26	+	28.64	YES
37	-	N/A	-	-	29.04	YES	-	-	28.14	YES
38	200x LOD	33,914	29.69	+	29.13	YES	28.69	+	28.53	YES
39	3x LOD	509	35.43	+	28.16	YES	34.32	+	28.09	YES
40	3x LOD	509	35.13	+	28.48	YES	34.13	+	28.11	YES

Table 27: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
41	-	N/A	-	-	28.31	YES	-	-	28.11	YES
42	2.25x LOD	382	35.31	+	28.28	YES	34.91	+	28.19	YES
43	-	N/A	-	-	28.66	YES	-	-	28.29	YES
44	-	N/A	-	-	28.47	YES	-	-	28.39	YES
45	200x LOD	33,914	29.04	+	28.17	YES	28.10	+	27.93	YES
46	-	N/A	-	-	28.51	YES	-	-	28.25	YES
47	-	N/A	-	-	28.31	YES	-	-	28.07	YES
48	2.25x LOD	382	35.39	+	28.85	YES	34.77	+	28.48	YES
49	2.25x LOD	382	35.77	+	28.51	YES	34.65	+	28.31	YES
50	-	N/A	-	-	28.41	YES	-	-	28.63	YES
51	3x LOD	509	35.56	+	28.31	YES	34.98	+	28.23	YES
52	3x LOD	509	35.19	+	28.18	YES	34.15	+	28.24	YES
53	200x LOD	33,914	29.20	+	28.30	YES	28.77	+	28.45	YES
54	-	N/A	-	-	28.23	YES	-	-	28.31	YES
55	-	N/A	-	-	29.63	YES	-	-	29.63	YES
56	200x LOD	33,914	29.36	+	28.62	YES	28.48	+	28.20	YES
57	-	N/A	-	-	28.38	YES	-	-	28.64	YES
58	-	N/A	-	-	28.28	YES	-	-	28.15	YES
59	3x LOD	509	34.71	+	28.62	YES	34.15	+	28.38	YES
60	-	N/A	-	-	28.35	YES	-	-	28.47	YES

Table 27: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
61	-	N/A	-	-	28.80	YES	-	-	29.96	YES
62	3x LOD	509	35.23	+	28.45	YES	35.18	+	29.39	YES
63	2.25x LOD	382	36.64	+	28.27	YES	36.52	+	29.61	YES
64	-	N/A	-	-	28.42	YES	-	-	29.33	YES
65	3x LOD	509	36.29	+	28.29	YES	34.86	+	28.89	YES
66	-	N/A	-	-	28.63	YES	-	-	28.54	YES
67	3x LOD	509	34.38	+	28.47	YES	35.66	+	29.08	YES
68	2.25x LOD	382	36.77	+	28.38	YES	35.83	+	29.66	YES
69	2.25x LOD	382	37.73	+	28.47	YES	35.20	+	29.15	YES
70	200x LOD	33,914	29.38	+	28.32	YES	30.11	+	29.64	YES
71	-	N/A	-	-	28.51	YES	-	-	28.65	YES
72	200x LOD	33,914	29.43	+	28.45	YES	29.14	+	28.69	YES
73	-	N/A	-	-	28.30	YES	-	-	28.60	YES
74	-	N/A	-	-	28.28	YES	-	-	29.23	YES
75	-	N/A	-	-	28.49	YES	-	-	29.27	YES
76	-	N/A	-	-	28.62	YES	-	-	29.11	YES
77	-	N/A	-	-	28.62	YES	-	-	29.31	YES
78	-	N/A	-	-	28.32	YES	-	-	29.09	YES
79	-	N/A	-	-	28.11	YES	-	-	29.04	YES
80	200x LOD	33,914	29.37	+	28.54	YES	29.00	+	28.77	YES

Table 27: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
81	-	N/A	-	-	28.40	YES	-	-	29.16	YES
82	-	N/A	-	-	28.31	YES	-	-	29.39	YES
83	200x LOD	33,914	29.30	+	28.08	YES	28.77	+	28.44	YES
84	-	N/A	-	-	28.55	YES	-	-	29.37	YES
85	-	N/A	-	-	28.54	YES	-	-	29.18	YES
86	200x LOD	33,914	29.32	+	28.42	YES	29.12	+	29.00	YES
87	2.25x LOD	382	36.09	+	28.62	YES	36.09	+	29.39	YES
88	2.25x LOD	382	35.21	+	28.17	YES	35.22	+	29.23	YES
89	-	N/A	-	-	28.41	YES	-	-	29.18	YES
90	3x LOD	509	35.56	+	28.38	YES	36.04	+	29.68	YES
91	-	N/A	-	-	28.28	YES	-	-	29.27	YES
92	-	N/A	-	-	28.28	YES	-	-	29.23	YES
93	2.25x LOD	382	38.39	+	28.39	YES	34.84	+	29.06	YES
94	-	N/A	-	-	28.21	YES	-	-	29.34	YES
95	200x LOD	33,914	30.76	+	29.97	YES	30.76	+	30.52	YES
96	-	N/A	-	-	30.04	YES	-	-	30.45	YES
97	3x LOD	509	36.54	+	28.39	YES	35.36	+	29.09	YES
98	-	N/A	-	-	28.15	YES	-	-	29.21	YES
99	-	N/A	-	-	28.13	YES	-	-	29.14	YES
100	3x LOD	509	36.70	+	29.38	YES	36.23	+	30.05	YES

Table 28: Mock Clinical Study on CFX96 Touch™ Deep Well Real-Time PCR Detection System - Summary Statistics

RealStar® MERS-CoV RT-PCR Kit U.S. in combination with the CFX96 Touch™ Deep Well Real-Time PCR Detection System	*upE*			*orf1a*		
	Positive results		Negative results	Positive results		Negative results
2.25x LOD (15 samples)	15		0	15		0
3x LOD (15 samples)	15		0	15		0
200x LOD (15 samples)	15		0	15		0
Neg. specimens (55 samples)	0		55	0		55
Total (100 samples)	45		55	45		55
			95% CI			95% CI
Positive Percent Agreement	45/45	100%	92.1% - 100%	45/45	100%	92.1% - 100%
Negative Percent Agreement	55/55	100%	93.5% - 100%	55/55	100%	93.5% - 100%

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96 Touch™ Deep Well Real-Time PCR Detection System correctly identified 45 of 45 specimens spiked with MERS-CoV RNA at the concentrations shown, including concentrations near the limit of detection of the assay. No unspiked specimen rendered a positive signal.

14.2 Mock Clinical Study - Nasopharyngeal Swabs

Data from the LoD study confirmed that in nasopharyngeal swab washes the LoD of the RealStar® MERS-CoV RT-PCR Kit U.S. for MERS-CoV is 55.2 PFU/mL. To predict clinical performance at the 95% confidence interval (CI), viral RNA at different concentrations was prepared, blinded and spiked into overall 45 samples of individual nasopharyngeal swab washes prepared as described in Section N-1. Fifteen specimens each were spiked at 2.25x LoD (124.2 PFU/mL), at 3x LoD (165.2 PFU/mL), and at a concentration of 200x LoD (11,040 PFU/mL). Another 50 individual nasopharyngeal swab washes were left unspiked. All samples were blinded, handed to an unbiased operator and extracted using the QIAamp® Viral RNA Mini Kit (QIAGEN). Eluates were analyzed with the RealStar® MERS-CoV RT-PCR Kit U.S. on the CFX96™/Dx Real-Time System (Bio-Rad). The blinded spiking key was unmasked after the results were complete. The results of the analyses can be found in the tables below.

Table 29: Mock clinical study: RealStar® MERS-CoV RT-PCR Kit U.S. on CFX96™/Dx Real-Time System

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
1	2.25x LoD	124.2	31.28	+	26.20	YES	30.9	+	26.16	YES
2	NTC	N/A	N/A	-	26.13	YES	N/A	-	26.16	YES
3	3x LoD	165.2	36.02	+	28.86	YES	32.27	+	28.84	YES
4	NTC	N/A	N/A	-	25.80	YES	N/A	-	26.10	YES
5	NTC	N/A	N/A	-	25.76	YES	N/A	-	26.04	YES
6	3x LoD	165.2	31.29	+	26.08	YES	30.75	+	26.13	YES
7	NTC	N/A	N/A	-	26.10	YES	N/A	-	26.45	YES
8	3x LoD	165.2	31.11	+	26.07	YES	30.82	+	26.16	YES
9	NTC	N/A	N/A	-	26.05	YES	N/A	-	26.08	YES
10	NTC	N/A	N/A	-	25.71	YES	N/A	-	26.03	YES
11	NTC	N/A	N/A	-	25.89	YES	N/A	-	26.08	YES
12	NTC	N/A	N/A	-	26.26	YES	N/A	-	26.26	YES
13	NTC	N/A	N/A	-	25.73	YES	N/A	-	26.07	YES
14	3x LoD	165.2	31.01	+	25.96	YES	30.85	+	26.35	YES
15	NTC	N/A	N/A	-	26.06	YES	N/A	-	26.15	YES
16	NTC	N/A	N/A	-	26.02	YES	N/A	-	26.31	YES
17	NTC	N/A	N/A	-	27.55	YES	N/A	-	27.45	YES
18	NTC	N/A	N/A	-	26.22	YES	N/A	-	26.08	YES
19	NTC	N/A	N/A	-	26.14	YES	N/A	-	26.19	YES
20	2.25x LoD	124.2	30.70	+	25.85	YES	30.92	+	26.05	YES

Table 29: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
21	3x LoD	165.2	31.04	+	26.15	YES	31.08	+	26.24	YES
22	NTC	N/A	N/A	-	25.74	YES	N/A	-	26.05	YES
23	2.25x LoD	124.2	31.07	+	25.94	YES	31.05	+	26.02	YES
24	NTC	N/A	N/A	-	26.21	YES	N/A	-	26.18	YES
25	NTC	N/A	N/A	-	25.74	YES	N/A	-	26.09	YES
26	NTC	N/A	N/A	-	25.85	YES	N/A	-	26.08	YES
27	2.25x LoD	124.2	31.12	+	25.91	YES	30.88	+	26.06	YES
28	NTC	N/A	N/A	-	25.66	YES	N/A	-	26.19	YES
29	200x LoD	11,040	26.92	+	28.70	YES	25.65	+	28.93	YES
30	NTC	N/A	N/A	-	25.82	YES	N/A	-	26.13	YES
31	200x LoD	11,040	25.09	+	25.83	YES	24.54	+	25.78	YES
32	200x LoD	11,040	24.70	+	25.69	YES	24.59	+	25.93	YES
33	NTC	N/A	N/A	-	26.11	YES	N/A	-	26.06	YES
34	200x LoD	11,040	25.74	+	26.82	YES	25.15	+	26.58	YES
35	NTC	N/A	N/A	-	25.63	YES	N/A	-	26.01	YES
36	NTC	N/A	N/A	-	26.07	YES	N/A	-	26.20	YES
37	200x LoD	11,040	24.91	+	25.90	YES	24.63	+	25.80	YES
38	NTC	N/A	N/A	-	26.05	YES	N/A	-	26.11	YES
39	NTC	N/A	N/A	-	26.00	YES	N/A	-	26.04	YES
40	3x LoD	165.2	31.30	+	26.01	YES	31.30	+	25.90	YES

Table 29: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
41	NTC	N/A	N/A	-	26.02	YES	N/A	-	26.14	YES
42	NTC	N/A	N/A	-	26.08	YES	N/A	-	26.20	YES
43	200x LoD	11,040	25.09	+	26.05	YES	24.82	+	25.83	YES
44	NTC	N/A	N/A	-	26.07	YES	N/A	-	26.29	YES
45	2.25x LoD	124.2	31.44	+	26.26	YES	31.08	+	26.12	YES
46	2.25x LoD	124.2	31.28	+	26.18	YES	31.14	+	26.21	YES
47	200x LoD	11,040	25.08	+	25.91	YES	24.68	+	25.95	YES
48	3x LoD	165.2	30.87	+	26.11	YES	31.1	+	26.27	YES
49	NTC	N/A	N/A	-	26.20	YES	N/A	-	26.35	YES
50	NTC	N/A	N/A	-	26.10	YES	N/A	-	26.19	YES
51	3x LoD	165.2	31.12	+	26.09	YES	31.23	+	26.33	YES
52	2.25x LoD	124.2	31.35	+	26.05	YES	31.30	+	26.36	YES
53	NTC	N/A	N/A	-	26.14	YES	N/A	-	25.98	YES
54	3x LoD	165.2	31.30	+	26.13	YES	30.81	+	26.18	YES
55	NTC	N/A	N/A	-	26.22	YES	N/A	-	26.26	YES
56	3x LoD	165.2	31.76	+	26.91	YES	31.53	+	27.04	YES
57	NTC	N/A	N/A	-	26.07	YES	N/A	-	26.33	YES
58	2.25x LoD	124.2	34.26	+	27.56	YES	32.30	+	27.73	YES
59	NTC	N/A	N/A	-	26.09	YES	N/A	-	26.26	YES
60	NTC	N/A	N/A	-	26.07	YES	N/A	-	26.31	YES

Table 29: (continuation)

Spiking key		Virus con. (PFU/ml)	*upE*				*orf1a*			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
61	2.25x LoD	124.2	31.2	+	26.23	YES	31.11	+	26.15	YES
62	NTC	N/A	N/A	-	26.27	YES	N/A	-	26.05	YES
63	2.25x LoD	124.2	31.67	+	26.29	YES	31.33	+	26.29	YES
64	200x LoD	11,040	25.18	+	25.96	YES	24.94	+	26.01	YES
65	NTC	N/A	N/A	-	26.15	YES	N/A	-	26.16	YES
66	200x LoD	11,040	30.23	+	33.13	YES	26.46	+	30.76	YES
67	NTC	N/A	N/A	-	26.11	YES	N/A	-	26.03	YES
68	3x LoD	165.2	31.13	+	25.82	YES	31.07	+	26.08	YES
69	2.25x LoD	124.2	34.08	+	29.28	YES	31.25	+	28.70	YES
70	NTC	N/A	N/A	-	25.55	YES	N/A	-	25.75	YES
71	NTC	N/A	N/A	-	25.57	YES	N/A	-	26.09	YES
72	200x LoD	11,040	27.37	+	29.25	YES	25.61	+	28.23	YES
73	2.25x LoD	124.2	33.95	+	29.11	YES	32.83	+	29.11	YES
74	NTC	N/A	N/A	-	25.98	YES	N/A	-	26.07	YES
75	200x LoD	11,040	25.07	+	25.91	YES	24.49	+	25.86	YES
76	2.25x LoD	124.2	30.88	+	25.96	YES	30.77	+	25.87	YES
77	NTC	N/A	N/A	-	25.95	YES	N/A	-	26.25	YES
78	200x LoD	11,040	28.01	+	30.01	YES	26.59	+	29.24	YES
79	NTC	N/A	N/A	-	25.61	YES	N/A	-	26.06	YES
80	NTC	N/A	N/A	-	26.03	YES	N/A	-	26.24	YES

Table 29: (continuation)

Spiking key		Virus con. (PFU/ml)	upE				orf1a			
			Cp value	Call	IC Cp	Agreement w/ expected result	Cp value	Call	IC Cp	Agreement w/ expected result
81	3x LoD	165.2	30.93	+	26.09	YES	31.01	+	26.18	YES
82	200x LoD	11,040	24.92	+	25.75	YES	24.57	+	25.77	YES
83	NTC	N/A	N/A	-	26.01	YES	N/A	-	26.07	YES
84	3x LoD	165.2	31.30	+	25.77	YES	30.27	+	25.95	YES
85	NTC	N/A	N/A	-	26.09	YES	N/A	-	26.17	YES
86	NTC	N/A	N/A	-	25.86	YES	N/A	-	26.14	YES
87	2.25x LoD	124.2	32.14	+	27.02	YES	31.36	+	26.04	YES
88	NTC	N/A	N/A	-	25.74	YES	N/A	-	25.94	YES
89	3x LoD	165.2	30.64	+	25.92	YES	30.46	+	26.05	YES
90	3x LoD	165.2	31.08	+	25.83	YES	30.93	+	26.12	YES
91	200x LoD	11,040	24.77	+	25.87	YES	24.72	+	26.04	YES
92	NTC	N/A	N/A	-	25.73	YES	N/A	-	26.04	YES
93	NTC	N/A	N/A	-	26.57	YES	N/A	-	26.29	YES
94	200x LoD	11,040	25.08	+	26.02	YES	24.57	+	25.59	YES
95	2.25x LoD	124.2	31.68	+	26.18	YES	31.29	+	26.31	YES

Table 30: Mock Clinical Study on CFX96 Touch™ Deep Well Real-Time PCR Detection System - Summary Statistics

RealStar® MERS-CoV RT-PCR Kit U.S. in combination with the CFX96 Touch™ Deep Well Real-Time PCR Detection System	upE			orf1a		
	Positive results		Negative results	Positive results		Negative results
2.25x LOD (15 samples)	15		0	15		0
3x LOD (15 samples)	15		0	15		0
200x LOD (15 samples)	15		0	15		0
Neg. specimens (50 samples)	0		50	0		50
Total (95 samples)	45		50	45		50
			95% CI			95% CI
Positive Percent Agreement	45/45	100%	92.1% - 100%	45/45	100%	92.1% - 100%
Negative Percent Agreement	50/50	100%	92.9% - 100%	50/50	100%	92.9% - 100%

The RealStar® MERS-CoV RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™/Dx Real-Time System correctly identified 45 of 45 specimens spiked with MERS-CoV RNA at the concentrations shown, including concentrations near the limit of detection of the assay. No unspiked specimen rendered a positive signal.

15. Quality Control

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

16. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: support@altona-diagnostics.com

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17. Trademarks and Disclaimers

RealStar® (altona Diagnostics GmbH); ABI Prism® (Applied Biosystems); LightCycler® (Roche); QIAamp® (QIAGEN); CFX96™ (BIO-RAD); VERSANT® (Siemens); Rotor-Gene® (Corbett Research); Rotor-Gene® (QIAGEN).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® MERS-CoV RT-PCR Kit U.S. is for use only under Emergency Use Authorization (EUA) by specified laboratories and clinical laboratory personnel who have been trained on authorized instruments.

Not available in all countries.

18. Explanation of Symbols

In vitro diagnostic medical device

For use only under Emergency Use Authorization

Product number

Batch code

Contains sufficient for “n” tests/reactions (rxns)


Temperature limitation


Version


Use until


Caution


Consult instructions for use


Manufacturer

Notes