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RealStar®

SARS-CoV-2 RT-PCR Kit U.S. Instructions for Use

For Use Under the Emergency Use Authorization (EUA) Only

Version 7.0 05/2020

RealStar®

RealStar®
SARS-CoV-2
RT-PCR Kit U.S.

For use with

CFX96™ Touch Real-Time PCR Detection 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
Rx only	For prescription use only
EUA	For use only under Emergency Use Authorization
REF	821025
Σ	384
	May 2020
	altona Diagnostics GmbH • Mörkenstr. 12 • D-22767 Hamburg

MAN-821020-EN-07

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1. Intended Use

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes and nasal aspirates from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. is intended for use by trained clinical laboratory personnel, specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The RealStar® SARS-CoV-2 RT-PCR Kit U.S. is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Background Information

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive sense, single stranded RNA virus belonging to the family *Coronaviridae*.

SARS-CoV-2 emerged in the Wuhan region of China in December 2019 and has spread worldwide within 2 months. The virus was initially termed as 2019-nCoV (novel Coronavirus) and renamed as SARS-CoV-2 by the “International Committee on Taxonomy of Viruses”, on 11.02.2020. At the same time the WHO named the disease, caused by SARS-CoV-2, COVID-19. Considering the rapid escalation and propagation of COVID-19 worldwide, the WHO characterized the outbreak as a pandemic on 12.03.2020.

SARS-CoV-2 is highly contagious and transmitted via aerosols and droplets and causes acute respiratory infections with flu-like symptoms. Mainly, but not exclusively, in elderly people and persons with pre-existing illness, infection with SARS-CoV-2 can lead to severe and life-threatening disease. Cases of asymptomatic infection, mild illness, severe illness, and deaths have been reported.

CAUTION	
	***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.***

3. Summary and Explanation

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. It is designed to detect RNA from the SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes and nasal aspirates from individuals who are suspected of COVID-19 by their healthcare provider.

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. includes two different amplification and detection systems. One primer and probe set enables the detection of an E gene sequence, which is specific for lineage B-betacoronavirus (B-βCoV; including SARS-CoV-2), whereas a second primer and probe set specifically amplifies and detects a SARS-CoV-2 specific sequence of the S gene.

In addition to the amplification and detection systems for lineage B-betacoronavirus and SARS-CoV-2 specific RNA the assay also includes a probe and primer set for the detection of an Internal Control to identify possible rRT-PCR inhibition and to confirm the integrity of the reagents of the kit.

The probe specific for lineage B-βCoV RNA (target E gene) is labelled with the fluorophore FAM™, whereas the SARS-CoV-2 RNA (target S gene) specific probe is labelled with the fluorophore Cy5. The probe specific for the target of the Internal Control (IC) is labelled with a fluorophore detectable in the VIC™ channel. Using probes linked to distinguishable dyes enables the parallel detection of lineage B-βCoV specific RNA, SARS-CoV-2 specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The workflow starts with collecting a nasal wash or nasal aspirate or with collecting a nasopharyngeal swab, oropharyngeal (throat) swab, anterior nasal or mid-turbinate nasal swab and placing this in Universal Transport Medium™, UTM® (Copan, Murrieta, California, USA). Nucleic acids are isolated and purified from the nasopharyngeal, oropharyngeal, anterior nasal or mid-turbinate nasal swab or from the nasal wash or aspirate using the following automated extraction system:

- AltoStar® Automation System AM16 in combination with the AltoStar® Purification Kit 1.5 (altona Diagnostics) and the AltoStar® Internal Control 1.5

The extraction of the RNA is performed following the manufacturer's instructions (for details, please refer to section 8.2). The extracted RNA will afterwards serve as template for analysis with the RealStar® SARS-CoV-2 RT-PCR Kit U.S.

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

- CFX96™ Touch Real-Time PCR Detection System (Bio-Rad, Cat. No. 1855195)
- CFX96™ Touch Deep Well Real-Time PCR Detection System (Bio-Rad, Cat. No. 185-4095)

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

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. contains of:

- Two Master reagents (Master A and Master B)
- Template Internal Control (IC)
- Positive Control
- Water (PCR grade)

Master A and Master B contain all components (buffer, enzymes, primers, and probes) to allow reverse transcription, PCR mediated amplification and target detection (SARS-CoV-2 specific RNA and Internal Control) in one reaction setup.

The following control materials are provided and to be used with the RealStar® SARS-CoV-2 RT-PCR Kit U.S.:

a) Internal Control (IC)

The Internal Control included in the RealStar® SARS-CoV-2 RT-PCR Kit U.S. used in preparation of Positive and Negative Controls for rRT-PCR and the AltoStar® Internal Control 1.5 that is added to each patient sample prior to extraction contain a defined copy number of an artificial DNA and an artificial RNA template molecule with no homologies to each other or to any other known sequences. Only the Internal Control RNA template is amplified and detected by the RealStar® SARS-CoV-2 RT-PCR Kit U.S. The DNA template is used on other RealStar® applications and plays no functional role in the RealStar® SARS-CoV-2 RT-PCR Kit U.S.

The AltoStar® Internal Control 1.5 is automatically added to the specimen/lysis buffer mixture at the beginning of the nucleic acid extraction procedure on the AltoStar® Automation System AM16. Both, the DNA and the RNA target molecules included in the AltoStar® Internal Control 1.5 are co-extracted with the nucleic acids included in the sample. Only the RNA IC target molecules are reverse transcribed, amplified and detected in parallel to the lineage B-betacoronavirus and SARS-CoV-2 specific RNA using the RealStar® SARS-CoV-2 RT-PCR Kit U.S.

The addition of 1 µl Internal Control provided with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. to the final rRT-PCR reaction for the Controls (i.e. Positive Control and Water (PCR grade)) leads to the same concentration of Internal Control RNA target molecules, which is present in the eluates from the nucleic acid extraction procedure using the AltoStar® Internal Control 1.5.

The function of the Internal Control is to ensure the integrity of the specific rRT-PCR results by indicating potential rRT-PCR inhibition.

b) Water (PCR grade)

The PCR grade water is to be used as “no template” (negative) control for the rRT-PCR reaction. Its function is to indicate contamination of rRT-PCR reagents. One no template control (Water (PCR grade)) is to be used per rRT-PCR run.

c) Positive Control

The Positive Control is needed to verify the functionality of the lineage B-betacoronavirus and SARS-CoV-2 RNA specific rRT-PCR amplification and detection systems included in the RealStar® SARS-CoV-2 RT-PCR Kit U.S. The Positive Control consists of a mixture of two *in vitro* transcripts (IVTs). One IVT contains a sequence of the E gene of lineage B-betacoronavirus, whereas the other IVT contains a sequence of the S gene of the SARS-CoV-2 genome. The E gene specific IVT and the S gene specific IVT contain the target region for the E gene and the S gene specific amplification and detection system, respectively. One positive template control (Positive Control) is to be used per rRT-PCR run.

d) Negative Process Control (NPC)

Apart from the controls provided with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. one Universal Transport Medium™ (UTM®, Copan, Murrieta, California, USA, or equivalent[1]) or saline (0.9%) sample should be included in each run (comprising extraction and rRT-PCR) as Negative Process Control (NPC) to monitor the nucleic acid extraction procedure.

4. Warnings and Precautions

- This assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization only.
- 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.
- Laboratories are required to report all positive results to the appropriate public health authorities.

[1] Because of shortages of specimen collection and transport devices, FDA is recommending alternative types of swab collection and transport media that are acceptable for use in testing for SARS-CoV-2. These recommendations are posted on FDA's FAQ website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2

- Results need to be interpreted in conjunction with clinical signs and symptoms of the patient or contact information.
- Do not use reagents from other manufacturers with this assay.
- Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.
- 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.
- 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.
- 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.
- Dispose sample and assay waste according to your local safety regulations.
- 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.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).

NOTE

Positive results are indicative of the presence of SARS-CoV-2 RNA and must be reported to the appropriate Public Health agency. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Please refer to the CDC website for the most update information on patient follow up: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html

Specimens tested positive only for the E gene are presumptive positive for SARS-CoV-2 RNA. Extraction and rRT-PCR analysis for such specimens should be repeated. In case of a repeatedly presumptive positive result, contact the responsible national reference center. Repeatedly presumptive positive results should be confirmed if clinically needed.

5. Principles of the Procedure

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® SARS-CoV-2 RT-PCR Kit U.S. is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. It is designed to detect RNA from the SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes and nasal aspirates from individuals who are suspected of COVID-19 by their healthcare provider.

One-step rRT-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.

6. Reagents

6.1 Kit Composition

Lid Color	Blue	Purple	Green	Red	White
Component	Master A	Master B	Internal Control	Positive Control	Water (PCR grade)
Number of Vials	8	8	4	2	2
Volume [µl/Vial]	240	720	1000	250	500
Main Ingredients	Oligo-nucleotides and dNTPs in buffered solution	DNA polymerase and reverse transcriptase in buffered solution	DNA and RNA template molecules with different sequences of artificial origin in buffered solution	Synthetic SARS-CoV-2 specific RNA in buffered solution	Nuclease-free water

6.2 Storage

- The RealStar® SARS-CoV-2 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 between -25°C and -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 between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

7. Specimen Collection and Preparation

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. For details, refer to the CDC guideline “Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)” (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).

NOTE

Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.

For details regarding nucleic acid extraction refer to section 8.2.

8. Procedure

8.1 Material and Devices required but not provided

- Appropriate real-time PCR instrument:
  - CFX96™ Touch Real-Time PCR Detection System (Bio-Rad, Cat. No. 1855195) - CFX Manager™ Software v3.1
  - CFX96™ Touch Deep Well Real-Time PCR Detection System (Bio-Rad, Cat. No. 185-4095) - CFX Manager™ Software v3.1
- Universal Transport Medium™, UTM® (Copan, Murrieta, California, USA, or equivalent[2]) for processing of swab specimens and for use as Negative Process Control (NPC)

[2] Because of shortages of specimen collection and transport devices, FDA is recommending alternative types of swab collection and transport media that are acceptable for use in testing for SARS-CoV-2. These recommendations are posted on FDA's FAQ website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2

- Appropriate nucleic acid extraction instrument, kit and/or reagents and disposables (refer also to section 8.2):
  - AltoStar® Automation System AM16 (altona Diagnostics, Cat. No. AM16) - AltoStar® Connect software version 1.7.4
  - AltoStar® Purification Kit 1.5 (altona Diagnostics, Cat. No. PK15-06)
  - AltoStar® Internal Control 1.5 (altona Diagnostics, Cat. No. IC15-06)
- 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)
- DNase/RNase-free pipette tips with aerosol barriers (disposable)
- Powder-free gloves (disposable)

8.2 RNA Extraction using the AltoStar® Automation System AM16

For the extraction of viral RNA using the AltoStar® Automation System AM16, 500 µL swab wash are used. Elution is performed with 80 µL elution buffer. During extraction the AltoStar® Internal Control is automatically added to the lysis buffer sample mixture.

Details on the RNA extraction process using the AltoStar® Automation System AM16 (altona Diagnostics) are given in Table 1. Carefully read the manufacturer's instructions for use for the AltoStar® Automation System AM16 and AltoStar® Purification Kit 1.5 for general handling instructions.

Table 1: Steps of the nucleic acid extraction procedure using the AltoStar® Automation System AM16

Step	Action
1. Start the AltoStar® AM16	• Switch on the AltoStar® AM16. • Switch on the computer and the monitor. • Start the AltoStar® Connect software.
2. Perform Maintenance	In the menu bar click **Application** → **Instrument Maintenance**. • If Weekly Maintenance is due, click **Start Weekly Maintenance.** • If Daily Maintenance is due, click **Start Daily Maintenance.** Follow the on screen instructions for the maintenance process.
3. Program an AltoStar® Run	In the menu bar click **Program Run** → **Program Run** (**AltoStar® Purification**). Alternatively, go back to the Start Screen and click the **Program Run** button. • Enter samples or import from LIMS, • Click the **Create Run** button in the tool bar to create the AltoStar® Run.

Step	Action
4. Start a Purification Run	In the menu bar click **Purification** → **Start Purification**. Alternatively, go back to the Start Screen and click the **Start Purification** button. • Select the Purification Run to be started to display the samples included in the selected Purification Run. • Prepare the purification reagents: ◦ Ensure that the purification reagents to be used have the same *Loading Number* (except AltoStar® Internal Control 1.5) and are not expired. ◦ If precipitates are visible in the Lysis Buffer, heat it (≤ 50 °C) until completely dissolved. ◦ Thaw the IC (AltoStar® Internal Control 1.5) and vortex for 5 seconds. ◦ Vortex the Magnetic Beads for 5 seconds without wetting the lid. • Prepare the samples for the Purification Run to be started as described in chapter 9.6.1 Sample Preparation in the manufacturer's instructions for use for the AltoStar® Purification Kit 1.5. • Click the **Start Run** button in the tool bar. • Follow the loading dialogs and load the instrument accordingly. • Confirm the **Loading Complete** message with **Ok** or wait 10 seconds. The system will now perform the Purification Run automatically.

Step	Action
5. Finish the Purification Run	• Make sure the Loading Tray is empty and confirm the **Run Finished** dialog with **Ok.** • Follow the instructions in the **Maintenance** dialog and confirm with **Ok.** • Seal and store the components of the AltoStar® Purification Kit 1.5 that can be reused. • If the PCR Setup is not performed right away, seal the Eluate Plate with the AltoStar® Eluate Plate Sealing Foil and store at 2 °C - 8 °C for up to 24 hours. • View the Purification Run results to confirm successful processing of each sample.

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

e-mail: **support@altona-diagnostics.com**
phone USA: **+1 614 706 178 4**
phone headquarter Hamburg: **+49 40 548 0676 0**

8.3 Master Mix Setup

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

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. contains a heterologous Internal Control (IC), which serves as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

The Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

8.4 Reaction Setup

- Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- Add 1 µl of the Internal Control provided with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. into the wells that will be used for the Negative Control (Water (PCR grade)) and the Positive Control. Do not add additional Internal Control template into the wells that will be used with any sample or control which has been extracted previously and already contains Internal Control.

- Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

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

8.5 Programming of Real-time PCR Instruments

For basic information regarding the setup and programming of the different CFX96™ real-time PCR instruments, please refer to the manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® SARS-CoV-2 RT-PCR Kit U.S. on the respective real-time PCR instruments please contact our Technical Support (see section 14).

8.5.1 Settings

- Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

8.5.2 Fluorescent Detectors (Dyes)

- Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter (Detection Channel)	Quencher
B-βCoV specific RNA	Target E gene	FAM™ (FAM™)	(None)
SARS-CoV-2 specific RNA	Target S gene	Cy5 (Cy5)	(None)
Internal Control	IC	JOE™ (VIC™)	(None)

8.5.3 Temperature Profile

- Define the temperature profile:

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

8.5.4 Special Remarks on the Setup of the CFX96™ Systems

The remarks on the setup refer to the following real-time PCR instruments:

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

Open the “Plate Setup” window and select “View / Edit Plate...”. Select all wells of the 96 well-plate. Click “Select Fluorophores”. For “Channel 1” check the box behind FAM™, for “Channel 2” check the box behind VIC™ and for “Channel 4” check the box behind Cy5. Assign samples to the wells by selecting the appropriate “Sample Type“ and afterwards “Load” FAM™, VIC™ and Cy5 to the wells. The target name of FAM™ should be set to “B-betaCoV E gene”, the target name of Cy5 should be set to “SARS-CoV-2 S gene” and the target name of VIC™ should be set to “Internal Control”.

9. Results

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® SARS-CoV-2 RT-PCR Kit U.S. on authorized real-time PCR instruments please contact our Technical Support (see section 14).

9.1 Validity of Diagnostic Test Runs

9.1.1 Valid Diagnostic Test Run

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

Control ID	FAM™ Detection Channel (E gene)	Cy5 Detection Channel (S gene)	VIC™ Detection Channel (Internal Control)
Positive Control*	C_t < 37	C_t < 37	C_t < 40
Negative Control (Water (PCR grade))	No C_t	No C_t	C_t < 40
Negative Process Control (NPC)	No C_t	No C_t	C_t < 40

* The Positive Control contains both targets, B-βCoV and SARS-CoV-2 specific RNA.

9.1.2 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 14).

9.2 Interpretation of Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If one or more controls are not valid, the patient results cannot be interpreted.

FAM™ (E gene)	Cy5 (S gene)	VIC™ (Internal Control)	Result Interpretation
C_t < 45	C_t < 45	Any or no C_t*	B-βCoV and SARS-CoV-2 specific RNA detected. Positive for SARS-CoV-2. Report result to healthcare provider and appropriate public health authorities.
C_t < 45	No C_t	Any or no C_t*	Only B-βCoV specific RNA detected. Presumptive positive for SARS-CoV-2. Repeat extraction and rRT-PCR. If the repeated result remains presumptive positive, contact the responsible national reference center. Repeated presumptive positive results should be confirmed if clinically needed.
No C_t	C_t < 45	Any or no C_t*	Only SARS-CoV-2 specific RNA detected. Positive for SARS-CoV-2. Report result to healthcare provider and appropriate public health authorities.
No C_t	No C_t	C_t < 40	Neither B-βCoV nor SARS-CoV-2 specific RNA detected. The sample does not contain detectable amounts of SARS-CoV-2 specific RNA. Report result to healthcare provider.
No C_t	No C_t	C_t > 40 or no C_t	rRT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the VIC™ detection channel can be reduced (i.e. C_t > 40) or absent (i.e. no C_t) due to a high SARS-CoV-2 RNA load in the sample.

NOTE

Positive results are indicative of the presence of SARS-CoV-2 RNA and must be reported to the appropriate Public Health agency. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Please refer to the CDC website for the most update information on patient follow up: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html

Specimens tested positive only for the E gene are presumptive positive for SARS-CoV-2 RNA. Extraction and rRT-PCR analysis for such specimens should be repeated. In case of a repeatedly presumptive positive result, contact the responsible national reference center. Repeatedly presumptive positive results should be confirmed if clinically needed.

10. Limitations

- Negative results do not preclude infection with SARS-CoV-2 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.
- Interpretation of rRT-PCR test results must account for the possibility of false negative and false positive results. False negative results can arise from:
  - poor sample collection or
  - degradation of the viral RNA during shipping or storage or
  - specimen collection conducted prior to symptom onset
  - failure to follow the authorized assay procedures
  - failure to use authorized extraction kit and instrument
- The performance of the RealStar® SARS-CoV-2 RT-PCR Kit U.S. was established using contrived nasopharyngeal swab samples. Nasal washes, nasal aspirates, anterior nasal swabs, mid-turbinate nasal swabs and

oropharyngeal (throat) swabs are also considered acceptable specimen types for use with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. but performance has not been established. Testing of anterior nasal and mid-turbinate nasal swabs (self collected or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to the FDA FAQs on Diagnostic Testing for SARS-CoV-2 (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2) for additional information regarding acceptable specimen types for detection of SARS-CoV-2.

- 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 SARS-CoV-2 vaccines and/or therapeutics on the ability to detect SARS-CoV-2 RNA in patient specimens has not been evaluated.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction using the AltoStar® Automation System AM16 must be conducted prior to using this assay.
- The presence of rRT-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.
- The E gene assay (FAM™ channel) does detect lineage B-betacoronavirus specific RNA including SARS coronavirus and several bat coronaviruses. Isolated signals with the E gene assay could indicate the presence of SARS coronavirus or bat coronaviruses.

11. Conditions of Authorization

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations

However, to assist clinical laboratories using the RealStar® SARS-CoV-2 RT-PCR Kit U.S. ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[3] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

[3] The letter of authorization refers to "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories".

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and altona Diagnostics (support@altona-diagnostics.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

G. altona Diagnostics, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

12. Performance Characteristics

12.1 Analytical Performance Evaluation

12.1.1 Analytical Sensitivity

Estimation of the Limit of Detection (LoD):
Serial dilutions of heat inactivated SARS-CoV-2 cell culture supernatant (4.6E+05 plaque forming units (PFU)/mL before inactivation; Institute of Virology, Charité Berlin, Germany) were used.

For extraction, 700 µL of UTM® containing simulated nasal matrix[4] were spiked with SARS-CoV-2 cell culture supernatant and loaded onto the AltoStar® Automation System AM16 for nucleic acid extraction with the AltoStar® Purification Kit 1.5 (volume used by the instrument for nucleic acid extraction was 500 µL). Each

[4] specimen contained simulated nasal matrix (5% w/v mucin, 5% v/v whole blood, 0.8% v/v NaCl (95% saline) and 0.00002% w/v human genomic DNA)

dilution was extracted in five replicates and tested with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. on the CFX96™ Touch Deep Well Real-Time PCR Detection System. The lowest concentration at which all replicates tested positive was treated as the estimated/tentative LoD. The results can be found in Table 2:

Table 2: Determination of the tentative LoD using the AltoStar® Automation System AM16 in combination with the AltoStar® Purification Kit 1.5 for nucleic acid extraction - Target: E gene

Target	Concentration [PFU/mL]	Call rate	Replicate 1 C_t (FAM™)	Replicate 2 C_t (FAM™)	Replicate 3 C_t (FAM™)	Replicate 4 C_t (FAM™)	Replicate 5 C_t (FAM™)
E gene	1.00E-01	5/5	32.79	33.30	33.03	33.24	33.14
	3.16E-02	4/5	-	35.23	35.25	39.43	34.22
	1.00E-02	4/5	-	35.68	38.77	36.25	36.10
	3.16E-03	2/5	-	-	-	38.30	38.70
	1.00E-03	0/5	-	-	-	-	-
	3.16E-04	0/5	-	-	-	-	-

Table 3: Determination of the tentative LoD using the AltoStar® Automation System AM16 in combination with the AltoStar® Purification Kit 1.5 for nucleic acid extraction - Target: S gene

Target	Concentration [PFU/mL]	Call rate	Replicate 1 C_t (Cy5)	Replicate 2 C_t (Cy5)	Replicate 3 C_t (Cy5)	Replicate 4 C_t (Cy5)	Replicate 5 C_t (Cy5)
S gene	1.00E-01	5/5	32.75	32.82	33.07	32.95	33.14
	3.16E-02	3/5	-	35.43	34.54	-	35.44
	1.00E-02	4/5	-	37.41	36.01	38.64	39.21
	3.16E-03	1/5	-	-	-	37.80	-
	1.00E-03	2/5	-	-	38.98	-	39.76
	3.16E-04	0/5	-	-	-	-	-

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. in conjunction with the AltoStar® Automation System AM16/AltoStar® Purification Kit 1.5 and the CFX96™ Touch Deep Well Real-Time PCR Detection System detected 5/5 replicates with a concentration of 1.00E-01 PFU/mL for both targets, the S gene and the E gene. Consequently, this concentration was considered the tentative LoD.

Confirmation of the Limit of Detection (LoD) using the CFX96™ Touch Deep Well Real-Time PCR Detection System for real-time RT-PCR:

Based on the tentative LoD, heat inactivated SARS-CoV-2 cell culture supernatant was spiked into 20 UTM® samples containing simulated nasal matrix to a final concentration of 1.00E-01 PFU/mL. Nucleic acids were extracted with the AltoStar® Purification Kit 1.5 on the AltoStar® Automation System AM16 as described above. The obtained eluates were tested with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. on the CFX96™ Touch Deep Well Real-Time PCR Detection System (Bio-Rad). The result can be found in Table 4:

Table 4: LoD confirmation on the CFX96™ Touch Deep Well Real-Time PCR Detection System

SARS-CoV-2 concentration = 1.00E-01 PFU/mL				
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (Cy5)**	**C_t (VIC™)**
1	Pos	33.02	33.04	29.57
2	Pos	33.28	33.29	29.34
3	Neg	-	-	29.25
4	Pos	33.34	33.76	29.45
5	Pos	32.82	33.88	29.42
6	Pos	32.79	32.85	29.61
7	Pos	32.43	33.53	29.53
8	Pos	33.14	33.26	29.47
9	Pos	33.01	32.68	29.45
10	Pos	33.2	33.45	29.31
11	Pos	33.21	33.51	29.41
12	Pos	32.99	34.11	29.56
13	Pos	32.69	33.13	29.41
14	Pos	33.67	34.33	29.52
15	Pos	32.55	32.76	29.48
16	Pos	33.26	33.32	29.33
17	Pos	33.2	32.53	29.36
18	Pos	32.78	33.00	29.51
19	Pos	33.13	33.31	29.47
20	Pos	33.28	33.43	29.46
Statistics	Mean C_t	33.04	33.32	29.45
	SD	0.30	0.47	0.09
	CV%	0.92	1.42	0.32
	Result	19/20		

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. in conjunction with the AltoStar® Automation System AM16/AltoStar® Purification Kit 1.5 and the CFX96™ Touch Deep Well Real-Time PCR Detection System detected 19/20 replicates at a concentration of 1.00E-01 PFU/mL. Therefore, the confirmed LoD is 1.00E-01 PFU/mL.

Confirmation of the Limit of Detection (LoD) using the CFX96™ Touch Real-Time PCR Detection System for real-time RT-PCR:

Based on the LoD determined for the RealStar® SARS-CoV-2 RT-PCR Kit U.S. using the CFX96™ Touch Deep Well Real-Time PCR Detection System (Bio-Rad), heat inactivated SARS-CoV-2 cell culture supernatant was spiked into 20 UTM® samples containing simulated nasal matrix to a final concentration of 1.00E-01 PFU/mL. Nucleic acids were extracted with the AltoStar® Purification Kit 1.5 on the AltoStar® Automation System AM16 as described above. The obtained eluates were tested with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. on the CFX96™ Touch Real-Time PCR Detection System (Bio-Rad). The result can be found in Table 5:

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

SARS-CoV-2 concentration = 1.00E-01 PFU/mL				
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (Cy5)**	**C_t (VIC™)**
1	Pos	32.58	33.58	29.89
2	Pos	32.41	33.26	29.97
3	Pos	32.85	34.72	29.93
4	Pos	32.67	33.71	29.86
5	Pos	33.14	34.25	29.94
6	Pos	32.79	33.83	29.69
7	Pos	32.74	34.64	30.00
8	Pos	32.91	34.48	29.97
9	Pos	32.90	34.30	30.03
10	Pos	32.69	35.95	29.93
11	Pos	32.62	34.28	29.78
12	Pos	33.13	35.08	29.69
13	Pos	33.48	33.74	29.82
14	Pos	33.62	33.54	29.90
15	Pos	33.03	34.22	29.78
16	Pos	32.91	35.09	30.01
17	Pos	32.54	33.91	29.72
18	Pos	33.48	35.51	30.01
19	Pos	33.16	34.82	29.97
20	Pos	32.58	33.82	29.77
Statistics	Mean C_t	32.91	34.34	29.88
	SD	0.34	0.70	0.11
	CV%	1.03	2.05	0.37
	Result		20/20	

The RealStar® SARS-CoV-2 RT-PCR Kit U.S. in conjunction with the AltoStar® Automation System AM16/AltoStar® Purification Kit 1.5 and the CFX96™ Touch Real-Time PCR Detection System detected 20/20 replicates at a concentration of 1.00E-01 PFU/mL. Therefore, the confirmed LoD is 1.00E-01 PFU/mL.

12.1.2 Analytical Specificity

12.1.2.1 Inclusivity

Inclusivity of the RealStar® SARS-CoV-2 RT-PCR Kit U.S. was evaluated for different isolates of SARS-CoV-2 by wet testing or *in silico* analysis. The results are shown in Table 6 and 7:

Table 6: Inclusivity (wet testing): RealStar® SARS-CoV-2 RT-PCR Kit U.S.

SARS-CoV-2 Strain/Isolate	Source/Sample Type	Concentration of the Stock before Heat Inactivation
*BetaCoV/Munich/ ChVir984/2020**	Institute of Virology; Charité Berlin; Germany/ Heat inactivated cell culture supernatant	4.6E+05 PFU/mL

* The strain *BetaCoV/Munich/ChVir984/2020* was used for determination of the LoD and the evaluation of the clinical performance of the RealStar® SARS-CoV-2 RT-PCR Kit U.S.

Table 7: Inclusivity (*In silico* analysis for 1906 whole genome sequences of SARS-CoV-2 of which 1809 were published via GISAID e.V. (www.gisaid.org) and 107 were published via the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) as of March 27, 2020 for the E gene and the S gene target): RealStar® SARS-CoV-2 RT-PCR Kit U.S.

1916 whole genome sequences		Homology	Comment
E gene	Forward Primer	1915 sequences: 100%	1 sequence 96% (1 mismatch)
	Reverse Primer	1915 sequences: 100%	1 sequence 95% (1 mismatch)
	Probe	1914 sequences: 100%	2 sequences: 95% (1 mismatch)
S gene	Forward Primer	1912 sequences: 100%	4 sequences: 95% (1 mismatch)
	Reverse Primer	1903 sequences: 100%	13 sequences: 95% (1 mismatch)
	Probe	1881 sequences: 100%	34 sequences: 95% (1 mismatch); 1 sequence: 91% (2 mismatches)

In a single oligonucleotide sequence mutation events leading to ≤ 2 mismatch/es will not have any significant negative impact on the amplification of the respective target sequence. None of the analyzed sequences showed mismatches in more than one oligonucleotide and none of the mismatching sequences showed mismatches with both specific detection systems (E gene and S gene), hence reactivity of the specific oligonucleotides included in the RealStar® SARS-CoV-2 RT-PCR Kit U.S. is not expected to be affected.

12.1.2.2 Cross Reactivity

To evaluate the analytical specificity of the RealStar® SARS-CoV-2 RT-PCR Kit U.S. with regards to cross-reactivity, genomic RNA or DNA from different viruses, bacteria and fungi as well as nucleic acid extracted from pooled nasal wash (for details refer to Table 8) was tested with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. on the CFX96™ Touch Deep Well Real-Time PCR Detection System (Bio-Rad).

RNA of the different coronaviruses (e.g. human coronavirus 229E, human coronavirus OC43, human coronavirus NL63, SARS-coronavirus and MERS-coronavirus) was tested directly in the PCR in the concentration indicated in Table 8 (10 µL of the RNA stock material was used in each PCR reaction).

For the other organisms, the nucleic acids (RNA or DNA) were extracted using the QIAamp® Viral RNA Mini Kit (RNA) or the QIAamp® DNA Mini Kit (DNA), respectively. The concentration indicated is the concentration of the stock used for the nucleic acid extraction. For the extraction using the QIAamp® Viral RNA Mini Kit an input volume of 140 µL of the stock was used. For the nucleic acid extraction using the QIAamp® DNA Mini Kit an input volume of 200 µL of the stock was used. In both cases the nucleic acids (RNA or DNA) were eluted in 60 µL. A total of 10 µL of the eluated nucleic acids were used in the PCR reaction.

Table 8: Cross Reactivity (wet testing): RealStar® SARS-CoV-2 RT-PCR Kit U.S.

Pathogen	Strain	Source/Sample type	Stock concentration	FAM™ channel (E gene)	Cy5 channel (S gene)	VIC™ channel (Internal Control)
Human coronavirus 229E	n.a.	EVA[5] / RNA	>1.00E+07 cp/mL	negative	negative	positive
Human coronavirus OC43	n.a.	EVA / RNA	740 ng/mL	negative	negative	positive
Human coronavirus NL63	n.a.	EVA / RNA	9.3E+08 cp/mL	negative	negative	positive
SARS-coronavirus[6]	n.a.	EVA / RNA	1.75E+08 cp/mL	positive	negative	positive
MERS-coronavirus	n.a.	EVA / RNA	1.02E+07 cp/mL	negative	negative	positive
Adenovirus	AV-1	ATCC[7] / DNA extracted with QIAamp® DNA Mini Kit	5.00E+6.5/mL	negative	negative	positive

[5] EVA: European Virus Archive

[6] It is correct that a positive result is obtained for SARS-coronavirus with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. in the FAM™ channel, since the E gene target is not SARS-CoV-2 specific, but detects all lineage B-betacoronaviruses including SARS-coronavirus.

[7] ATCC: American Type Culture Collection

Table 8: (continuation)

Pathogen	Strain	Source/Sample type	Stock concentration	FAM™ channel (E gene)	Cy5 channel (S gene)	VIC™ channel (Internal Control)
Human Metapneumovirus (hMPV)	IA27-2004	ZeptoMetrix / RNA extracted with QIAamp® RNA Viral Kit	1.00E+6.1/ml (TCID50)	negative	negative	positive
Parainfluenza virus 1	1	ATCC / RNA extracted with QIAamp® RNA Viral Kit	4.04E+06 cp/mL	negative	negative	positive
Parainfluenza virus 2	2	ATCC / RNA extracted with QIAamp® RNA Viral Kit	4.49 E+08 cp/mL	negative	negative	positive
Parainfluenza virus 3	3	ATCC / RNA extracted with QIAamp® RNA Viral Kit	1.04E+07 cp/mL	negative	negative	positive
Parainfluenza virus 4	4b	ATCC / RNA extracted with QIAamp® RNA Viral Kit	1.80E+09 cp/mL	negative	negative	positive

Table 8: (continuation)

Pathogen	Strain	Source/Sample type	Stock concentration	FAM™ channel (E gene)	Cy5 channel (S gene)	VIC™ channel (Internal Control)
Influenza A virus	Virginia	ATCC / RNA extracted with QIAamp® RNA Viral Kit	6.1E+06 PFU/mL	negative	negative	positive
Influenza B virus	Tai-wan/2/62	ATCC / RNA extracted with QIAamp® RNA Viral Kit	3.2E+07 PFU/mL	negative	negative	positive
Enterovirus	EV68	ATCC / RNA extracted with QIAamp® RNA Viral Kit	1.00E+5.5 /ml (TCID50)	negative	negative	positive
Respiratory syncytial virus A	A	ATCC / RNA extracted with QIAamp® RNA Viral Kit RNA	680 ng/mL	negative	negative	positive
Respiratory syncytial virus B	B	ZeptoMetrix / RNA extracted with QIAamp® RNA Viral Kit	1E+6.66/mL (TCID50)	negative	negative	positive
Rhinovirus	RV42	Public Health England / RNA extracted with QIAamp® RNA Viral Kit	No data available	negative	negative	positive

Table 8: (continuation)

Pathogen	Strain	Source/Sample type	Stock concentration	FAM™ channel (E gene)	Cy5 channel (S gene)	VIC™ channel (Internal Control)
Chlamydia pneumoniae	TWAR-183	DSMZ[8] / DNA extracted with QIAamp® DNA Mini Kit	1.00E+04 cp/mL	negative	negative	positive
Haemophilus influenzae	n.a.	DSMZ / DNA extracted with QIAamp® DNA Mini Kit	1.00E+05 cp/mL	negative	negative	positive
Legionella pneumophila	n.a.	DSMZ / DNA extracted with QIAamp® DNA Mini Kit	1.00E+05 cp/mL	negative	negative	positive
Streptococcus pneumoniae	SF 130, T1	DSMZ / DNA extracted with QIAamp® DNA Mini Kit	1.00E+05 cp/mL	negative	negative	positive
Streptococcus pyogenes	n.a.	DSMZ / DNA extracted with QIAamp® DNA Mini Kit	1.00E+05 cp/mL	negative	negative	positive

[8] DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen

Table 8: (continuation)

Pathogen	Strain	Source/Sample type	Stock concentration	FAM™ channel (E gene)	Cy5 channel (S gene)	VIC™ channel (Internal Control)
Bordetella pertussis	18323	DSMZ / DNA extracted with QIAamp® DNA Mini Kit	1.00E+05 cp/mL	negative	negative	positive
Mycoplasma pneumoniae	522	DSMZ / DNA extracted with QIAamp® DNA Mini Kit	1.00E+05 cp/mL	negative	negative	positive
Pneumocystis jirovecii (PJP)	FH	ATCC / DNA extracted with QIAamp® DNA Mini Kit	1.00E+04 cp/mL	negative	negative	positive
Candida albicans	NIH 3172	ATCC / DNA extracted with QIAamp® DNA Mini Kit	No data available	negative	negative	positive
Pseudomonas aeruginosa	Migula	ATCC / DNA extracted with QIAamp® DNA Mini Kit	1.83E+07 CFU/mL	negative	negative	negative
Pooled human nasal wash	n.a.	Pool prepared with nasal swabs in UTM® from 5 single donors	No data available	negative	negative	positive

No cross-reactivity of the RealStar® SARS-CoV-2 RT-PCR Kit U.S. with genomic RNA/DNA of the selected pathogens and with nucleic acid extracted from pooled nasal wash was observed. All samples tested generated a positive Internal Control signal in the VIC™ channel, whereas with the exception of SARS-coronavirus[9] no signal was observable in the E gene target specific FAM™ channel and in the S gene target specific Cy5 channel.

For all pathogens included in the wet testing as well as for additional pathogens with limited or no availability an *in silico* analysis was performed showing that cross-reactivity with the primers and probes included in the RealStar® SARS-CoV-2 RT-PCR Kit U.S. is unlikely to occur.

In silico analysis included genomic RNA/DNA sequences of the viruses and organisms listed in Table 9.

[9] It is correct that a positive result is obtained for SARS-coronavirus with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. in the FAM™ channel, since the E gene target is not SARS-CoV-2 specific, but detects all lineage B-betacoronaviruses including SARS-coronavirus.

Table 9: Cross Reactivity (*in silico* analysis): RealStar® SARS-CoV-2 RT-PCR Kit U.S.

Viruses and Organisms	
SARS-coronavirus	*Chlamydia pneumoniae*
Human coronavirus 229E	*Haemophilus influenzae*
Human coronavirus OC43	*Legionella pneumophila*
Human coronavirus HKU1	*Mycobacterium tuberculosis*
Human coronavirus NL63	*Streptococcus pneumoniae*
MERS-coronavirus	*Streptococcus pyogenes*
Adenovirus	*Bordetella pertussis*
Human metapneumovirus (hMPV)	*Mycoplasma pneumoniae*
Parainfluenza virus 1-4	*Pneumocystis jirovecii* (PJP)
Influenza A virus	*Candida albicans*
Influenza B virus	*Pseudomonas aeruginosa*
Enterovirus	*Staphylococcus epidermidis*
Respiratory syncytial virus	*Streptococcus salivarius*
Rhinovirus	---

The sequences of the primers and probes included in the RealStar® SARS-CoV-2 RT-PCR Kit U.S. were blasted against the species specified in Table 9 above.

The search parameters used for BLAST analysis were set to: max target sequences: 20000, short queries, automatically adjust parameters for short input sequences, expect threshold: 10, word size: 16, max matches in a query range: 0, match/ mismatch scores: 2,-3, low complexity regions filter. 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.

12.2 Clinical Performance Evaluation

To predict clinical performance at the 95% confidence interval (CI), SARS-CoV-2 cell culture supernatant at different concentrations was prepared, blinded and spiked into overall 34 individual nasopharyngeal swabs resuspended in Universal Transport Medium™ (UTM®). Ten specimens each were spiked with RNA at a final concentration of the 1x LoD (1.00E-01 PFU/mL), fourteen specimens each were spiked with RNA at a final concentration of the 2x LoD (2.00E-01 PFU/mL), and ten specimens each were spiked with RNA at a final concentration of the 20 x LoD (2.00E00 PFU/mL). Another 35 individual presumed SARS-CoV-2 negative nasopharyngeal swabs resuspended in Universal Transport Medium™ (UTM®) were left unspiked. All samples were blinded, handed to an unbiased operator. Nucleic acids were extracted using the AltoStar® Automation System AM16 in combination with the AltoStar® Purification Kit 1.5 (altona Diagnostics). Eluates were tested with the RealStar® SARS-CoV-2 RT-PCR Kit U.S. on the CFX96™ Touch Deep Well Real-Time PCR Detection System (Bio-Rad). The blinded spiking key was unmasked after the results were complete. The results are shown in Table 10:

Table 10: Results from testing clinical samples

Sample Concentration [PFU/mL]	Call Rate Target S gene	Call Rate Target E gene
1x LoD (1.00E-01)	9/10	10/10
2x LoD (2.00E-01)	14/14	14/14
20x LoD (2.00E00)	10/10	10/10
negative	0/35	0/35

95% (23/24) of the samples with a SARS-CoV-2 concentration of the 1x or the 2x LoD were tested positive for the S gene target and were reported as “Positive for SARS-CoV-2 RNA”. One sample was positive only for the E gene target and was reported as “Presumptive positive for SARS-CoV-2 RNA”. All (100%) of these samples were tested positive for the E gene target. From the samples with a concentration of the 20x LoD all (100%) were tested positive for the S gene as well as for the E gene target. All unspiked samples (100%) were tested negative for both targets.

13. Quality Control

In accordance with the altona Diagnostics GmbH DIN EN ISO 13485-certified Quality Management System, each lot of RealStar® SARS-CoV-2 RT-PCR Kit U.S. is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: support@altona-diagnostics.com

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15. Trademarks and Disclaimers

AltoStar®, RealStar® (altona Diagnostics); CFX96™, CFX96 Manager™ (Bio-Rad); QIAamp® (QIAGEN); FAM™, ROX™, VIC™, JOE™ (LifeTechnologies); Universal Transport Medium™, UTM® (Copan).

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® SARS-CoV-2 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.

16. Explanation of Symbols

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

always a drop ahead.

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