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artus® SARS-CoV-2 Prep&Amp™ UM Kit Instructions for Use (Handbook)

Σ 768

Σ 3072

Version 1

IVD

For In Vitro Diagnostic Use on Rotor Gene® Q MDx 5plex HRM or ABI® 7500 Fast Dx instruments

REF 4511460, 4511469

QIAGEN, GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

R1

Sample to Insight

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Intended Use

The *artus* SARS-CoV-2 Prep&Amp UM Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs (NPS) from individuals with signs and symptoms of infection, or individuals without symptoms or other reasons to suspect COVID-19 infection.

It is intended as an aid in the diagnosis of COVID-19 in the acute phase of infection in combination with clinical observations, patient history, and epidemiological information.

The *artus* SARS-CoV-2 Prep&Amp UM Kit is to be used in a molecular biology laboratory environment by professional users, such as trained clinical laboratory personnel specifically instructed in the techniques of real-time RT-PCR and in vitro diagnostic procedures.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.

The *artus* SARS-CoV-2 Prep&Amp UM Kit is intended to be used with the Rotor-Gene Q MDx System or ABI 7500 Fast Dx real-time PCR systems.

Description and Principle

Pathogen information

Coronaviruses, a genus in the family Coronaviridae, are large enveloped, positive-stranded RNA viruses that cause highly virulent disease in humans and domestic animals (1). Coronaviruses are known to infect humans account for one-third of common cold infections and are also a well-known cause of nosocomial upper respiratory infections in premature infants (2).

A novel member of the coronavirus family caused an outbreak of the respiratory disease in Wuhan City in China (1, 1). First named novel coronavirus (2019-nCoV), SARS-CoV-2 differs from SARS-CoV (1, 1), which was responsible for the 2003 outbreak, and MERS CoV, which has been circulating in the Middle East since 2012. SARS-CoV-2 is the causative agent of COVID-19. SARS-CoV-2 RNA is detectable during the early and acute phases of the infection from nasopharyngeal swabs.

Combined with patient history and SARS-CoV-2 epidemiology, real-time RT-PCR assays have become the gold standard for SARS-CoV-2 diagnosis. The European Centre for Disease Prevention and Control (ECDC) has proposed to combine real-time RT-PCR-based assays with immunoassays to monitor infection status and to evaluate the efficiency of the restrictive measures taken to control the outbreak (3, 4).

The *artus* SARS-CoV-2 Prep&Amp UM Kit is designed to cover 2 targets (N1 and N2) of the N gene detected with the same fluorescence channel. The two targets are not differentiated, and amplification of either or both targets leads to a fluorescence signal. Positive results are indicative of the presence of SARS-CoV-2 but do not rule out co-infection with other pathogens. On the other hand, negative real-time RT-PCR results do not exclude a possible infection.

Summary and Explanation

The *artus* SARS-CoV-2 Prep&Amp UM Kit constitutes a ready-to-use system with a simple sample preparation step followed by detection of the SARS-CoV-2 RNA using real-time RT-PCR on the RGQ MDx system or ABI 7500 Fast Dx (Figure 1).

The SARS-CoV-2 UM Amp Buffer contains reagents and enzymes for the specific amplification of a 72 base pair (bp) and a 67 bp region of the SARS-CoV-2 RNA genome and for their direct detection in the "Green" fluorescence channel of the RGQ MDx instruments and in the "FAM" fluorescence channel of the ABI 7500 Fast Dx.

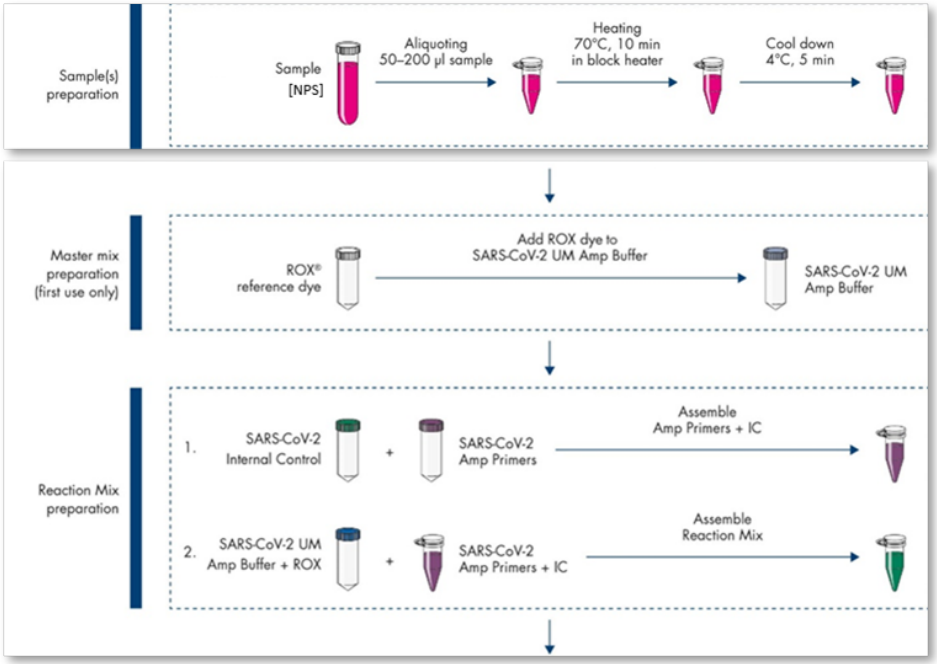
The Primers and Probes Mix of the *artus* SARS-CoV-2 Prep&Amp UM Kit also contains the oligonucleotides required for the RNase P amplifications. When detected in the "Yellow" fluorescence channel of the RGQ MDx instrument and in the VIC/HEX of ABI 7500 Fast Dx, those amplifications assure that enough biological sample has been collected. This control is critical to ensure the presence of biological samples in SARS-CoV-2 negative samples. An amplification should always be detectable; otherwise, it questions the sample quality.

The *artus* SARS-CoV-2 Prep&Amp UM Kit also contains a third heterologous amplification system to reveal possible real-time RT-PCR inhibition. This is detected as an internal RNA control (IC) in the "Red" fluorescence channel of the RGQ MDx instruments or in the Cy5/ATTO647N of ABI 7500 Fast Dx. Because the IC is included in the SARS-CoV 2 Amp Primers Mix, its amplification should be constant unless a real-time RT-PCR inhibitor is present in the sample or in the PCR reaction, which delays or prevents amplification.

External positive and negative controls (SARS-CoV-2 Positive Control and nuclease-free water used as NTC, respectively) are supplied in the artus SARS-CoV-2 Prep&Amp UM Kit to attest of the performance of the PCR step. A no extraction control (SARS-CoV-2 UM Prep Buffer used as

NEC) is strongly recommended to verify the absence of real-time RT-PCR inhibitors in the preparation buffer.

Taken together, the efficiency of the reverse transcription and the PCR steps are monitored by these controls.



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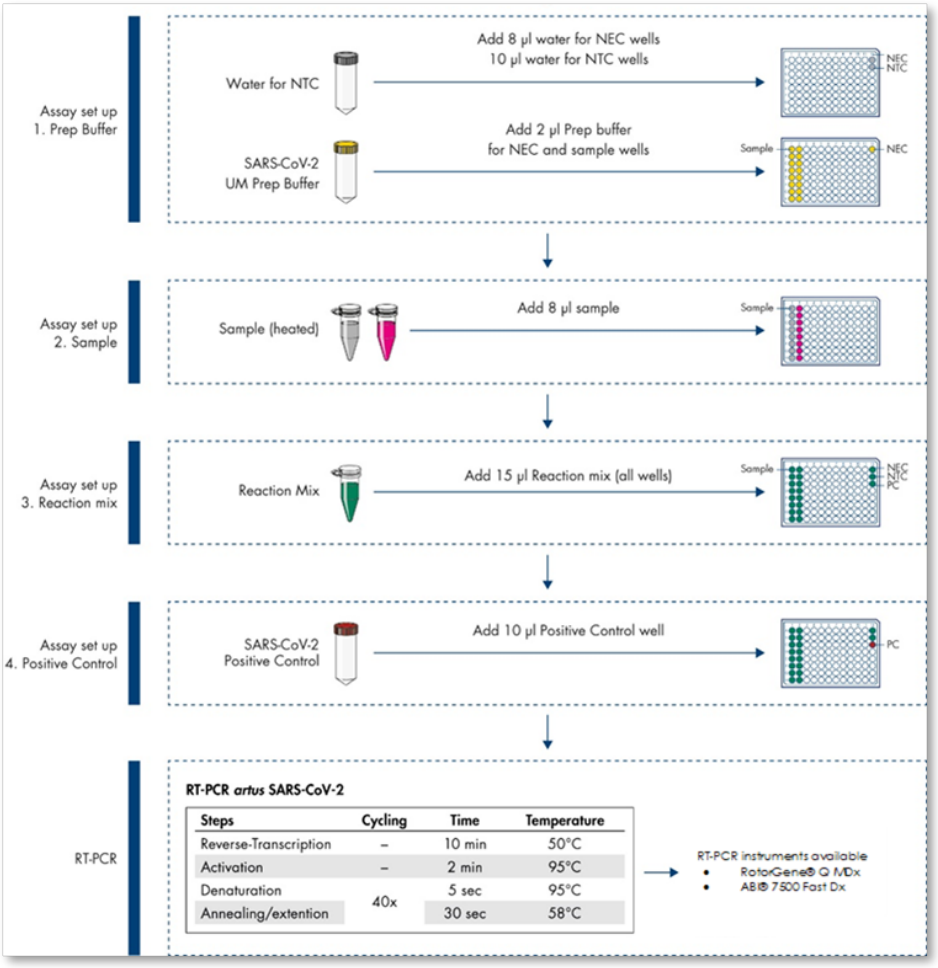

Figure 1. *artus* SARS-CoV-2 Prep&Amp UM Kit workflow.

Materials Provided

Kit Contents

***artus* SARS-CoV-2 Prep&Amp UM Kit** **Catalog no.** **Number of reactions**				**4511460** **768**	**4511469** **3072**
Tube color	Lid Color	Identity	Tube ID	Volume (µL)	Volume (µL)
Clear	Yellow	SARS-CoV-2 UM Prep Buffer	Preparation Buffer	2 x 930	8 x 930
Clear	Blue	SARS-CoV-2 UM Amp Buffer	Master Mix	4 x 1440	16 x 1440
Clear	Purple	SARS-CoV-2 Amp Primers	Primers and Probes	4 x 1680	16 x 1680
Clear	Green	SARS-CoV-2 Internal Control	Internal Control (IC)	1 x 1390	4 x 1390
Clear	Red	SARS-CoV-2 Positive Control	Positive Control	1 x 220	4 x 220
Clear	Clear	Water for NTC	Water (NTC)	1 x 1900	4 x 1900
Clear	Clear	ROX Reference Dye	ROX Dye	1 x 210	4 x 210

Kit components

Reagents

In each tube, the reagent volumes have been optimized for 8 batches of 96 samples (for the 768 reactions kit) or 32 batches of 96 reactions (for the 3072 reactions kit), including a positive control (PC), a no template control (NTC), and a no extraction control (NEC).

Fewer or a greater number of samples may be run, but there will be sub-optimal reagent usage. It is recommended to avoid multiple freeze–thaw cycles. Reagents may be aliquoted to avoid multiple freeze–thaw cycles.

Primers and probes

Primers and probes targeting the SARS-CoV-2 sequences are based on the primers and probes designed by the Centers for Disease Control and Prevention (CDC).

Controls and calibrators

The assay contains 5 controls to monitor the real-time RT-PCR efficiency.

Internal control (IC): The internal control is a single-strand IVT RNA that verifies the presence of contaminants that could inhibit the reverse transcription. The internal control also monitors the reverse transcription efficiency in the no template control (NTC) and in the no extraction control (NEC).

No template control (NTC): The no template control is composed of nuclease-free water. It is added to the PCR plate to verify introduction of contaminants during the PCR plate preparation that could lead to misinterpretation of the SARS-CoV-2 targets.

Positive control (PC): The positive control is a double-strand DNA amplified with the SARS CoV 2 Primers and Probes (P&P mix). Its detection verifies the efficiency of the reagent involved in the PCR amplification step.

No extraction control (NEC): The no extraction control is composed of the SARS-CoV-2 UM Prep Buffer. It is processed in parallel with the clinical samples to verify introduction of contaminants during the sample preparation that could lead to misinterpretation of the SARS CoV-2 targets.

Sampling Control: The Sampling Control detects the RNase P gene and is critical to ensure the presence of biological samples in SARS-CoV-2 negative samples. Amplification of the sampling control should always be detectable; otherwise, it questions the sample quality.

Platforms and software

Prior to use, ensure that instruments have been maintained and calibrated according to the manufacturer's recommendations. This kit can be used in five workflows that require the use of the following real-time RT-PCR instruments and their appropriate software:

- Rotor-Gene Q MDx 5plex HRM: Rotor-Gene Q software version 2.3.1 or higher
- ABI 7500 Fast Dx: SDS software version 1.4.1 or higher

Materials Required but Not Provided

Common consumables and equipment

- Desktop centrifuge with rotor for 2 mL reaction tubes
- Pipettes (adjustable)
- Vortex mixer
- Block heater
- Disposable powder-free gloves
- Sterile and nuclease-free pipette tips with filters
- 1.5 mL or 2 mL PCR-free tubes
- 96-well plate centrifuge instrument

Consumables and equipment for each platform

Rotor-Gene Q MDx 5plex HRM Instrument

- 0.1 mL PCR tubes, for use with the Rotor-Gene Q MDx (Strip Tubes and Caps, 0.1 mL, cat. no. 981103).
- 72-Well Rotor (cat.no. 9018903) and Locking Ring 72-Well Rotor (cat. no. 9018904)

ABI 7500 Fast Dx Instrument

96-Well MicroAmp™ (Thermo Fisher Scientific, cat. no. N8010560)

MicroAmp Optical Adhesive film (Thermo Fisher Scientific, cat. no. 4360954)

Warnings and Precautions

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and/or its authorized representative and the regulatory authority in which the user and/or the patient is established.

The clinical performance for SARS-CoV-2 has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in a convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Always wear appropriate personal protective equipment, including but not limited to disposable powder-free gloves, a lab coat, and protective eyewear. Protect skin, eyes, and mucus membranes. Change gloves often when handling samples.

All samples should be treated as potentially hazardous. Always observe safety precautions as outlined in relevant guidelines, such as the Clinical and Laboratory Standards Institute® (CLSI) *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline* (M29), or other appropriate documents.

Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

Precautions

- Observe standard laboratory procedures for keeping the working area clean and contamination-free. Dedicate an area with specific equipment to manipulate RNA.
- Follow good laboratory practices to minimize cross-contamination.
- Pay attention to avoid contamination with RNase during the experiment and use RNase free plasticware.
- Make sure to have a good traceability with records, especially for sample identification.

Reagent Storage and Handling

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

The *artus* SARS-CoV-2 Prep&Amp UM Kit can be kept at –30°C to –15°C until expiry date.

Once opened, reagents can be stored in their original packaging at –30°C to –15°C for 10 months or until the stated expiration date, whichever comes first. Repeated freezing and thawing should be avoided. A maximum of four freeze-thaw cycles is recommended.

Specimen Transport, Storage, and Handling

The *artus* SARS-CoV-2 Prep&Amp UM Kit is for use with nasopharyngeal swabs. All samples should be treated as potentially hazardous. The Centers for Disease Control and Prevention (CDC) and Public Health England have provided guidelines for sample collection, handling, and testing clinical specimens. Refer to these guidelines or to other relevant national reference laboratory protocols for additional information.

Nasopharyngeal swab collection, transport and storage

For swab collection, storage, and transport, please refer to the supplier's recommendations. Swabs must be fully immersed in transport media to maintain specimen integrity. Nasopharyngeal swab samples remain stable and can be stored at:

- 4°C (2 to 8°C) for up to 72 hours
- –70°C for 2 weeks

Nasopharyngeal swab samples remain stable through 3 freeze–thaw cycles.

Protocol: Sample preparation and SARS-CoV-2 Detection on the RGQ MDx 5plex HRM

This protocol describes the sample and the real-time RT-PCR preparation to detect the SARS CoV-2 targets in human nasopharyngeal swabs stored in transport media on the RGQ MDx 5plex HRM real-time RT-PCR instrument associated with the Rotor-Gene Q software version 2.3.1.49 (or higher).

Important points before starting

- Verify that the expiration dates and storage conditions printed on the box and all component labels are followed. Do not use expired or incorrectly stored components.
- Use well-maintained and calibrated equipment.
- Pay attention to avoid contamination with RNases during the experiment and use nuclease free plasticware.

Things to do before starting

- Respiratory samples may be kept at room temperature (15–25°C) during preparation steps and reaction setup, but it is recommended to keep them on ice or at 4°C on a cooling rack.
- Before use, let the SARS-CoV-2 UM Prep Buffer, SARS-CoV-2 UM Amp Buffer, SARS- CoV-2 Amp Primers, SARS-CoV-2 IC, Water for NTC, and SARS-CoV-2 Positive Control completely thaw at room temperature. Keep tubes at room temperature and protected from light until use.
- Before use, homogenize the SARS-CoV-2 UM Prep Buffer and the SARS-CoV-2 UM Amp Buffer by inverting them 2-3 times (do not vortex), followed by a quick spin. All the other

individual reagents can be homogenized by pulse vortexing for 3-5 seconds or by inverting 2-3 times, followed by a quick spin.

- The SARS-CoV-2 UM Prep Buffer inhibits RNases present in the clinical samples for the detection step, but it is not a virus-inactivating solution. All samples should be treated as potentially hazardous.
- Verify that the cycling conditions of the real-time RT-PCR platform are as specified in this protocol.
- Reagents may be aliquoted to avoid multiple freeze–thaw cycles.
- Freshly prepare the reaction mix (<2 h to the RT-PCR plate launch).
- To minimize contamination, the sample and the RT-PCR preparations should be done in distinct zones.

Procedure

Sample preparation: For nasopharyngeal swabs, follow Step 1.

1. Nasopharyngeal swabs:
    a. Vortex the swab containing the sample vigorously.
    b. Aliquot 50-200 µL of sample into 1.5mL PCR-free tubes
    c. Perform heating step at 70°C for 10 min on a block heater. Cool down the samples on ice for at least 5 min. then, keep the samples on ice or at 4°C.
2. At first use, complete the SARS-CoV-2 UM Amp Buffer with the ROX reference dye.
    a. Add 32.8 µL of the ROX dye to 1 tube of SARS-CoV-2 UM Amp Buffer.
    b. Close the lid containing the SARS-CoV-2 UM Amp Buffer and the ROX dye and invert the tube 3 times.

c. Spin down the SARS-CoV-2 UM Amp Buffer containing ROX dye at the bottom of the tube.

3. For a full RGQ MDx plate (72 wells), prepare an aliquot mix of the SARS-CoV-2 Amp Primers with the SARS-CoV-2 Internal Control.

   a. Transfer the required volumes of the SARS-CoV-2 Amp Primers and the SARS-CoV-2 Internal Control according to Table 1 into a new 1.5 mL PCR-free tube.

   b. Close the lid and invert the tube 3 times or pulse vortex the tube for 3-5 s.

   c. Spin down the SARS-CoV-2 Amp Primers containing the IC at the bottom of the tube.

Table 1. SARS-Cov-2 Amp Primers + IC Mix Setup

SARS-CoV-2 Amp Primers + IC mix				**Number of reactions Volume (µL)**
Reagents	Stock concentration	Final concentration	1 rxn	72 rxns (+20% extra volume*)
SARS-CoV-2 Amp Primers	3.45x	1x	7.25	626.4
SARS-CoV-2 Internal Control	166.67 cp/µL	10 cp/µL	1.5	129.6
Total SARS-CoV-2 Amp Primers + IC mix			8.75	756

Note: Adjust the volumes of SARS-CoV-2 Amp Primers and SARS-CoV-2 Internal Control according to the number of samples to be tested. Consider extra volume to compensate for the dead volume.

4. Prepare a reaction mix according to Table 2 and mix thoroughly by inverting the tube 3 times.

Table 2. Reaction Mix Setup

RT-PCR reaction mix			Number of reactions Volume (µL)	
Reagents	Stock concentration	Final concentration	1 rxn	72 rxns (+20% extra volume)
SARS-CoV-2 UM Amp Buffer +ROX mix	4x	1x	6.25	540
SARS-CoV-2 Amp Primers + IC mix	2.9x	1x	8.75	756
Total reaction volume			15.00	1296

Note: Adjust the volumes of SARS-CoV-2 Amp Buffer and SARS-CoV-2 Amp Primers according to the number of samples to be tested. Consider extra volume to compensate for the dead volume.

5. Dispense 8 µL of nuclease-free water to the PCR tube assigned to the NEC.
6. Load 10 µL of nuclease-free water into the PCR tube assigned to the NTC.
7. Dispense 2 µL of SARS-CoV-2 UM Prep Buffer into each PCR tube assigned to the NEC and the prepared samples.
8. Add 8 µL of the prepared sample to a PCR tube containing the SARS-CoV-2 UM Prep Buffer. Mix by pipetting up and down 5 times.
9. Add 15 µL of the reaction mix prepared in Step 5 to the tubes dedicated to samples and controls (Figure 2 provided as an example). Mix by pipetting up and down 5 times, then close the PCR tube lids, except for the one reserved as the SARS-CoV-2 Positive Control.

   Note: Verify that tubes are well closed to prevent cross-contamination.
10. Load 10 µL of the SARS-CoV-2 Positive Control into the appropriate PCR tube. Mix by pipetting up and down 5 times.
11. Set the RT-PCR program of the RGQ MDx 5plex HRM according to specifications in Table 3.

    Note: Data acquisition should be performed during the annealing/extension step.

12. Place tubes in the real-time cycler (an example of tube layout is represented in Figure 2), and start the cycling program as described in Table 3.

Note: Be careful to follow the same tube position and order between the assay set-up and the real-time cycler steps.

Table 3. SARS-CoV-2 Prep&Amp UM Program

Steps	Time	Temperature (°C)	Number of cycles	Acquisition
Reverse transcription	10 min	50	1	No
PCR initial heat activation	2 min	95	1	No
2-step cycling				
Denaturation Annealing/Extension	5 s 30 s	95 58	40	No Green , Yellow and Red

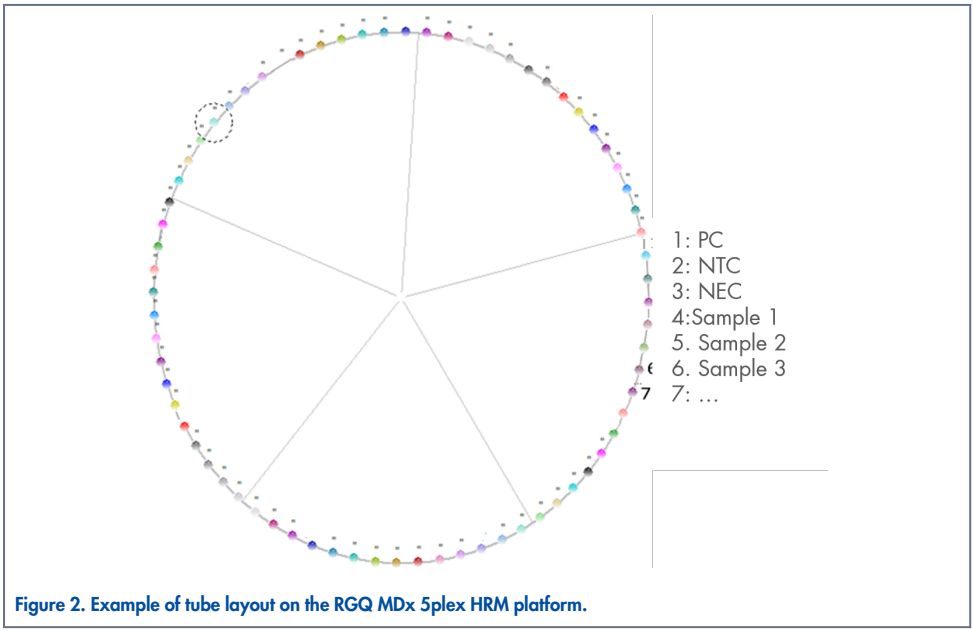

Figure 2. Example of tube layout on the RGQ MDx 5plex HRM platform.

13. Click Gain optimization in the "New Run Wizard" and open Auto-gain Optimization Setup.

14. Verify that the acquisition channels are set as described in Table 4.

Table 4. RGQ MDx 5plex HRM configuration

Name	PC tube position	Min reading (Fl)	Max reading (Fl)	Min gain	Max gain
Green	1*	5 Fl	10 Fl	–10	10
Yellow	1*	5 Fl	10 Fl	–10	10
Red	1*	5 Fl	10 Fl	–10	10

Note: This needs to be changed according to the SARS-CoV-2 Positive Control tube position.

15. Select Perform optimization before the first acquisition.

16. Start the run.

17. At the end of the run, analyze results (see the Results section).

Protocol: Sample preparation and SARS-CoV-2 Detection on ABI 7500 Fast Dx

This protocol is for preparing and detecting SARS-CoV-2 targets in human nasopharyngeal swabs stored in transport media on the ABI 7500 Fast Dx real-time RT-PCR instrument.

Important points before starting

- Verify that the expiration dates and storage conditions printed on the box and all component labels are followed. Do not use expired or incorrectly stored components.
- Use well-maintained and calibrated equipment.
- Pay attention to avoid contamination with RNases during the experiment, and use nuclease free plasticware.
- When using ABI 7500 Fast Dx, ROX Dye must be added to the master mix tube before first use.

Things to do before starting

- Respiratory samples may be kept at room temperature (15–25°C) during preparation steps and reaction setup, but it is recommended to keep them on ice or at 4°C on a cooling rack.
- The ROX dye is required when using the ABI 7500 Fast Dx.
- Data must be acquired with the ROX passive dye setting.
- Before use, let the SARS-CoV-2 UM Prep Buffer, SARS-CoV-2 UM Amp Buffer, SARS-CoV 2 Amp Primers, SARS-CoV-2 IC, Water for NTC, and SARS-CoV-2 Positive Control completely thaw at room temperature. Keep tubes at room temperature and protected

from light until use.

- Before use, homogenize the SARS-CoV-2 UM Prep Buffer and the SARS-CoV-2 UM Amp Buffer by inverting 2-3 times (do not vortex), followed by a quick spin. All the other individual reagents can be homogenized by pulse vortexing for 3-5 seconds or by inverting 2-3 times, followed by a quick spin.
- The SARS-CoV-2 UM Prep Buffer inhibits RNases present in the clinical samples for the detection step but is not a virus-inactivating solution. All samples should be treated as potentially hazardous.
- Verify that the cycling conditions of the real-time RT-PCR platform are as specified in this protocol.
- Reagents may be aliquoted to avoid multiple freeze–thaw cycles.
- Freshly prepare the reaction mix (<2 h to the RT-PCR plate launch).
- To minimize contamination, the sample and the RT-PCR preparations should be done in distinct zones.

Procedures

Sample preparation: For nasopharyngeal swabs, follow Step 1.

1. 1. Nasopharyngeal swabs:
    a. Vortex the swab containing sample vigorously.
    b. Aliquot 50-200 µl of sample into 1.5mL PCR-free tubes.
    c. Perform heating step at 70°C for 10 min on a block heater.
    d. Cool down samples on ice for at least 5 min, then keep the samples on ice or at 4°C.
2. At first use, complete the SARS-CoV-2 UM Amp Buffer with the ROX Reference Dye.

a. Add 32.8 µL of the ROX Dye to a tube of SARS-CoV-2 UM Amp Buffer.

b. Close the lid containing the SARS-CoV-2 UM Amp Buffer and the ROX Dye and invert the tube 3 times.

c. Spin down the SARS-CoV-2 UM Amp Buffer containing ROX Dye at the bottom of the tube.

3. For a full ABI 7500 Fast Dx plate (96 wells), prepare an aliquot mix of the SARS-CoV-2 Amp Primers with the SARS-CoV-2 Internal Control.

   a. Transfer the required volume of the SARS-CoV-2 Amp Primers and the SARS-CoV-2 Internal Control according to Table 5 into a new 1.5 mL PCR-free tube.

   b. Close the lid and invert the tube 3 times or pulse vortex the tube for 3-5 s.

   c. Spin down the SARS-CoV-2 Amp Primers containing the IC to bring the solution to the bottom of the tube.

Table 5. SARS-CoV-2 Amp Primers + IC mix setup

SARS-CoV-2 Amp Primers + IC mix			Number of reactions Volume (µL)	
Reagents	**Stock concentration**	**Final concentration**	**1 rxn**	**96 rxns (+ 20% extra volume*)**
SARS-CoV-2 Amp Primers	3.45x	1x	7.25	835.2
SARS-CoV-2 Internal Control	166.67 cp/µL	10 cp/µL	1.5	172.8
Total SARS-CoV-2 Amp Primers + IC mix			8.75	1008

Note: Adjust the volumes of SARS-CoV-2 Amp Primers and SARS-CoV-2 Internal Control according to the number of samples to be tested. Consider extra volume to compensate for the dead volume.

4. Prepare a reaction mix according to Table 6 and mix thoroughly by inverting the tube 3 times.

Table 6. Reaction mix setup

RT-PCR reaction mix			Number of reactions Volume (µl)	
Reagents	Stock concentration	Final concentration	1 rxn	96 rxns (+20% extra volume*)
SARS-CoV-2 UM Amp Buffer + ROX mix	4x	1x	6.25	720
SARS-CoV-2 Amp Primers + IC mix	2.9x	1x	8.75	1008
Total reaction volume	–		15.00	1728

Note: Adjust the volume of SARS-CoV-2 UM Amp Buffer and SARS-CoV-2 Amp Primers according to the number of samples to test. Consider extra volume to compensate for the dead volume.

5. Dispense 8 µL of nuclease-free water to the well assigned to the NEC.
6. Load 10 µL of nuclease-free water into the well assigned to the NTC.
7. Dispense 2 µL of SARS-CoV-2 UM Prep Buffer into each well assigned to the NEC and to the prepared samples.
8. Add 8 µL of the prepared sample to a well containing the SARS-CoV-2 UM Prep Buffer. Mix by pipetting up and down 5 times.
9. Add 15 µL of the reaction mix prepared in Step 5 to the wells dedicated to samples and controls (see example on Figure 3). Mix by pipetting up and down 5 times.
10. Load 10 µl of the SARS-CoV-2 Positive Control into the appropriate well. Mix by pipetting up and down 5 times.
11. Seal the PCR plate well to prevent cross-contamination. Make sure to apply pressure uniformly across the entire plate to obtain a tight seal across individual wells.
12. Centrifuge the PCR plate briefly to collect liquid at the bottom of the well.

13. Set the real-time RT-PCR program on the "Standard 7500" Run Mode of the ABI 7500 Fast Dx according to Table 7.

    Note: After clicking on **file** and **new**, verify that the assay is **Standard Curve (Absolute Quantitation)**, and Run Mode is set to **Standard 7500**. Select the FAM, VIC, and Cy5 as reporters with Quencher set to **None**, and data must be acquired with the **ROX** as **passive reference**.

    Note: Data acquisition should be performed during the annealing/extension step.

    Note: Please refer to the ABI 7500 Fast Dx Instructions for Use for more details.

14. Place the plate in the real-time cycler (an example of a PCR plate layout is represented in Figure 3 and start the cycling program as described in Table 7.

15. Select the used wells and apply the FAM, VIC, and Cy5 reporters. Data must be acquired with the ROX passive dye ON.

16. Verify that the Standard Curve of the ABI 7500 Fast Dx is configured to Absolute Quantitation.

17. Start the run.

18. At the end of the run, analyze results (see the Results section).

Table 7. SARS-CoV-2 Prep&Amp UM program

Steps	Time	Temperature (°C)	Number of cycles	Acquisition
Reverse transcription	10 min	50	1	No
PCR initial heat activation	2 min	95	1	No
2-step cycling				
Denaturation Annealing/Extension	5 s 30 s	95 58	40	No FAM, VIC, and Cy5

	1	2	3	4	5	6	7	8	9	10	11	12
A	*PC*											
B	*NTC*											
C	*NEC*											
D	*Sample 1*											
E	*Sample 2*											
F	*Sample 3*											
G	...											
H												

Figure 3. Example of plate layout on ABI 7500 Fast Dx.

Results

Analysis on RGQ MDx 5plex HRM

On the RGQ MDx 5plex HRM, the data are analyzed with the Rotor-Gene Q software version 2.3.1 (or higher) according to the manufacturer's instructions (Rotor-Gene Q MDx User Manual, Revision 7, September 2018).

For data analysis, the crop cycle must be used (Figure 4): Open the Raw Channel **Cycling A.Green**. Go to **Options** > **Crop Start Cycles** and Enter **5** in the dialog box. A new channel will be generated named Cycling A(from 5).Green. The same must be done for raw channels Red and Yellow to generate channels **Cycling A(from 5)**.**Red** and **Cycling A(from 5).Yellow**.

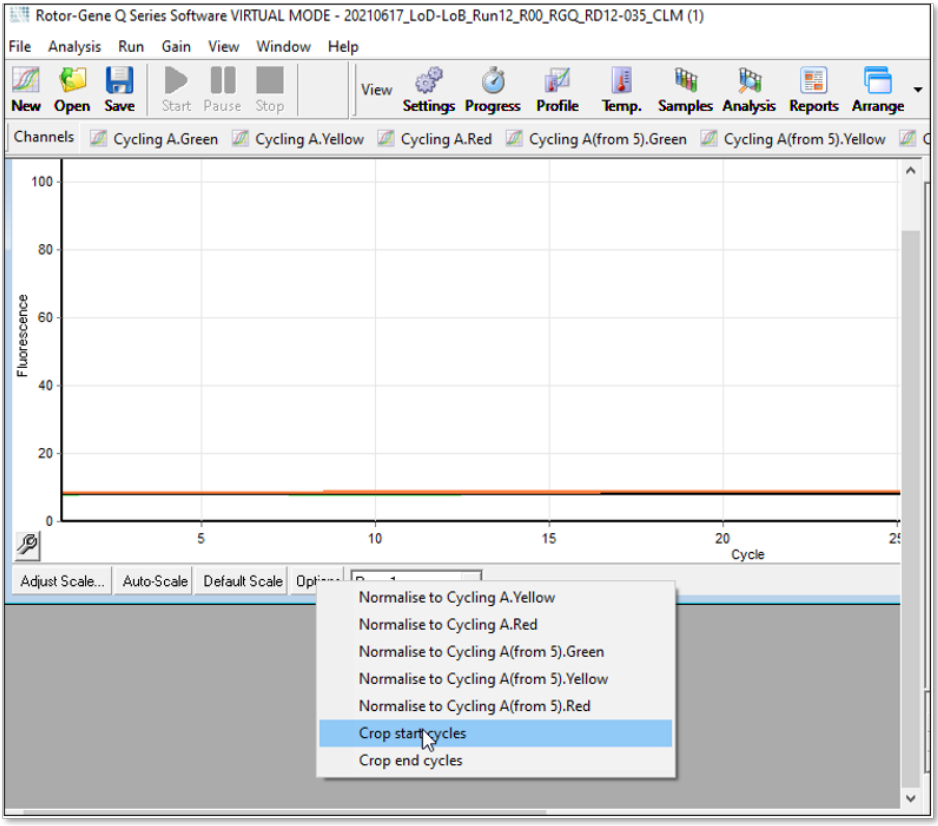

Figure 4. Screenshot of setting crop cycles for the RGQ MDx 5plex HRM runs analysis.

Open the analysis menu (Figure 5), and for each generated channel **Cycling A(from 5)**, apply the following analysis parameters to maintain consistency between different analyses (Table 9).

Table 8. Analysis parameters for the RGQ MDx 5plex HRM

Channels	Green	Red	Yellow
Fluorescence threshold	0.03	0.03	0.03
Slope correction	Yes	Yes	Yes
Dynamic tube	Yes	Yes	Yes
Take-off point	No	10-20	10-20
Outlier Removal: Reaction Efficiency Threshold	Yes Enabled 0%	No	Yes Enabled -85%
Cropped start cycles	5	5	5
Cut-off cycles	Ct >38.00 is considered as 40.00	No	Ct >35.00 is considered as 40.00

In the RGQ software, run results are available in the quantitation results grid opened during the analysis. Data can be exported in comma separated value text (.csv) format: In the RGQ Software window, select **File** > **save as** > **Excel analysis sheet** . Make sure that all samples are selected before exporting the results (Figure 5).

Figure 5. Screenshot of selected channels for applying the analysis parameters and exporting results (the RGQ MDx 5plex HRM runs analysis).

Analysis on ABI 7500 Fast Dx

On the ABI 7500 Fast Dx, the data are analyzed with the 7500 Fast System Software version 1.4.1 (or higher) according to the manufacturer's instructions. In the **setup** tab, select group of wells or the entire plate available in the analysis and right click to open the well inspector windows. The 3 fluorophores (FAM, VIC, and Cy5) must be selected, and **ROX** must be selected as **Passive reference**. The following parameters are needed for consistency between different analyses (Table 8).

Table 9. Analysis parameters for the ABI 7500 Fast Dx

Channels	FAM	Cy5	VIC
Passive dye	ROX	ROX	ROX
Fluorescence threshold	0.13	0.025	0.05
Baseline set	Auto	Auto	Auto
Cut-off cycles	Ct >39.00 is considered as 40.00	No	Ct > 35.00 is considered as 40.00

In the ABI SDS software, Ct values of a selected group of wells or the entire plate are available in the data sheet of the Results main section. Data can be exported in comma separated value text (.csv) format: In the SDS Software window, select **File** > **Export** > **Results** (menu item **Ct** can also be chosen). Select the format of the exported file as **.csv**.

Interpretation of Results

The positive control (PC), the N1, and the N2 genes are detected in the Green fluorescence channel with the RGQ MDx 5plex HRM or in the fluorescent channel FAM on the ABI 7500 Fast Dx.

The sampling control, composed of the RNase P, is detected in the Yellow fluorescence channel with the RGQ MDx 5plex HRM or in the fluorescence VIC/HEX with the ABI 7500 Fast Dx. Every clinical sample should display a sampling control amplification. In the PC, a Yellow amplification may be seen despite the absence of human sequences. In this case, a signal in the PC Yellow channel may be ignored because the strong fluorescence signal in the Green channel may bleed in the Yellow channel. The internal control (IC) is included in the SARS-CoV-2 Amp Primers. It is detected in the no template control (NTC), the no extraction control (NEC), the positive control (PC) and the clinical samples with the Red fluorescence channel with the RGQ MDx 5plex HRM or in the fluorescence channel Cy5/ATTO647N with the ABI 7500 Fast Dx. For a real-time RT PCR run to be valid, a PC, NTC, and NEC controls must perform as shown in (Table 10, Table 11).

Table 10. Run validity criteria and result interpretation for the RGQ MDx 5plex HRM

Control	Detection in Green channel	Detection in Yellow channel	Detection in Red channel	Interpretation
Positive control (PC)	Ct ≤ 38.00	Indifferent	Indifferent	PC is valid.
	Ct > 38.00 or No Ct	Indifferent	Indifferent	PC is invalid.
No template control (NTC) or	Ct > 38.00 or No Ct	Ct > 35.00 or No Ct	Yes	NTC/NEC is valid.
No extraction control (NEC)	Any other combinations with amplification in Green or Yellow		Indifferent	NTC/NEC is invalid.

Table 11. Run validity criteria and result interpretation for the ABI 7500 Fast Dx, real-time RT-PCR instrument

Control	Detection in FAM dye	Detection in VIC/HEX dye*	Detection in Cy5/ATTO647N dye*	Interpretation
Positive control (PC)	Ct ≤ 39.00	Indifferent	Indifferent	PC is valid.
	Ct > 39.00 or No Ct	Indifferent	Indifferent	PC in invalid.
No template control (NTC) or No extraction control (NEC)	Ct > 39.00 or No Ct	Ct > 35.00 or No Ct	Yes	NTC/NEC is valid.
	Any other combinations with amplification in FAM or VIC/HEX		Indifferent	NTC/NEC is invalid.

To validate the tested samples, the samples must be amplified and detected as expected.

Table 12. Sample validity criteria and results interpretation for the RGQ MDx 5plex HRM

Detection in Green channel	Detection in Yellow channel	Detection in Red channel	Interpretation
Ct ≤ 38.00	Indifferent	Indifferent	Sample is positive for SARS-CoV-2 RNA.
Ct > 38.00 or No Ct	Ct ≤ 35.00	Indifferent	Sample is negative, SARS-CoV-2 RNA is not detected.
Ct > 38.00 or No Ct	Ct > 35.00 or No Ct	Yes	Invalid sample. No or insufficient human material detected. Re-sampling is required.
Ct > 38.00 or No Ct	Ct > 35.00 or No Ct	No	Invalid sample. Real-time RT-PCR reaction is inhibited. A retest is required.

Table 13. Sample validity criteria and results interpretation for the ABI 7500 Fast Dx real-time RT-PCR instrument

Detection in FAM dye*	Detection in VIC/HEX dye*	Detection in Cy5/ATTO647N dye*	Interpretation
Ct ≤ 39.00	Indifferent	Indifferent	Sample is positive.
Ct > 39.00 or No Ct	Ct ≤ 35.00	Indifferent	Sample is negative, SARS-CoV-2 is not detected.

Table 13. Sample validity criteria and results interpretation for the ABI 7500 Fast Dx real-time RT-PCR instrument (continued)

Detection in FAM dye*	Detection in VIC/HEX dye*	Detection in Cy5/ATTO647N dye*	Interpretation
Ct > 39.00 or No Ct	Ct > 35.00 or No Ct	Yes	Invalid sample. No human material detected. Re-sampling is required.
Ct > 39.00 or No Ct	Ct > 35.00 or No Ct	No	Invalid sample. Real time RT-PCR reaction is inhibited. A retest is required.

Limitations

- For in vitro diagnostic use only.
- Results from the *artus* SARS-CoV-2 Prep&Amp UM Kit are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results do not preclude infection with SARS-CoV-2 and should not be the sole basis of a patient treatment decision.
- The product is to be used by personnel especially instructed and trained in in vitro diagnostics procedures.
- Strict compliance with the real-time RT-PCR platform's user manual (Rotor-Gene Q 5-plex HRM MDx or ABI 7500 Fast Dx) is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- To avoid the risk of having a false-negative result in case low positive clinical sample is tested when blood traces are observed in the tube, this should be recorded, and if the sample returns a negative result when using the *artus* SARS-CoV-2 Prep&Amp UM Kit, the sample should be re-collected from the patient and should be tested again with the artus SARS-CoV-2 Prep&Amp UM Kit.

Performance Characteristics

Analytical sensitivity (Limit of detection)

The analytical sensitivity, or the limit of detection, is defined as the lowest concentration at which ≥95% of the tested samples generate a positive call. The LoD was assessed by analyzing serial dilutions of negative nasopharyngeal samples prepared with high-titer stocks of inactivated viral particles obtained from commercial suppliers (ZeptoMetrix®). Two sample pools were used for each specimen for the LoD experiments. To confirm the established LoD concentration, the detection rate of all replicates must be ≥95% (at least 19/20 replicates must generate a positive signal).

The LoD concentration was verified on nasopharyngeal specimens on the claimed real time RT-PCR platforms (RGQ MDx and ABI 7500 Fast Dx).

Nasopharyngeal samples

The LoD is claimed at 950 cp/mL for the RGQ MDx and ABI 7500 Fast Dx (see Table 14)

Table 14. LoD results summary for each real time RT-PCR platform

Platform	Specimen type	LoD verified (cp/mL)
RGQ MDx	NPS	950
ABI 7500 Fast Dx	NPS	950

Analytical specificity studies (Inclusivity and exclusivity/cross-reactivity)

Inclusivity

The inclusivity of the *artus* SARS-CoV-2 Amp Primers and Probes has been assessed with an in silico analysis on sequences available in GISAID database (**www.gisaid.org**). A total of 722,488 sequences (available at the 23/03/2021) were analyzed on COVID CG (**https://covidcg.org**), alimented by GISAID metadata. Sequences were aligned to the WIV04 reference sequence (100% identical to Wuhan-Hu-1/NC_045512.2, except for the length of the poly-A tail) and the single nucleotide variations (SNVs) were analyzed in the genomic region targeted by the *artus* SARS- CoV- 2 Prep&Amp UM Kit Primers and Probes. The prevalence of the identified SNVs stayed below 1%, as well as the frequency of the co occurring mutations. There was no SNV located at the last 1 to 3 nucleotides from the 3' end in the respective oligonucleotides, which would be expected to impact performance. The *artus* SARS CoV-2 Prep&Amp UM Kit is considered able to detect 100% of the published sequences.

Exclusivity/Cross-reactivity

In silico analysis

The exclusivity of the *artus* SARS-CoV-2 Amp Primers and Probes has been assessed with an in silico analysis on sequences stored in the NCBI databank. The in silico analysis showed that some of the tested pathogens have more than 80% homology with one of the artus SARS CoV 2 primers or probes. Among these are Candida albicans, SARS-CoV-1, Streptococcus pyogenes, and Streptococcus salivarius. Pseudomonas aeruginosa had less than 80% homology with one of the primers/probes of the SARS-CoV-2 assay. However, the *artus* SARS CoV-2 Amp Primers and Probes showed no possible amplification with the different sequences stored in the NCBI nr/nt database.

A total of 36 bacterial, viral, and fungal strains (Table 15) have been analyzed by in silico PCR with a limited potential amplicon size of 500 bp. Pathogen sequences were collected from the NCBI database, however, none of these pathogens showed amplification in silico. Table 15 shows the list of pathogen tested in silico.

Table 15. List of in silico tested pathogens.

Pathogens	Strain/Type	Taxonomy ID	In silico PCR results
Adenovirus Type 3	Type 3	45659	No match
Adenovirus Type 4	Type 4	28280	No match
Adenovirus Type 5	Type 5	28285	No match
Adenovirus Type 7A	Type 7A	85755	No match
Adenovirus Type 14	Type 14	10521	No match
Adenovirus Type 31	Type 31	10529	No match
Bordetella pertussis	A639	520	No match
Candida albicans	Z006 SC5314	5476	No possible amplification*†
Chlamydia pneumoniae	CWL-029 TW-183	115713	No match
Enterovirus	Type 68	42789	No match
Haemophilus influenzae	KW20	727	No match
Human coronavirus	229E	11137	No match
Human coronavirus	NL63	277944	No match
Human coronavirus	HKU-1	290028	No match
Human coronavirus OC43	OC43	31631	No match
Human Coronavirus	MERS-CoV	1335626	No match
Human Metapneumovirus	n/a	162145	No match
Influenza A	H1N1	114727	No match
Influenza A	H3N2	119210	No match

Table 15. List of in silico tested pathogens. (continued)

Pathogens	Strain/Type	Taxonomy ID	In silico PCR results
Influenza B	n/a	11520	No match
Mycoplasma pneumoniae	M129 FH	272634	No match
Parainfluenza virus	Type 1	12730	No match
Parainfluenza virus	Type 2	2560525	No match
Parainfluenza virus	Type 3	11216	No match
Parainfluenza virus	Type 4	2560526	No match
Pneumocystis jirovecii	RU7	42068	No match
Pseudomonas aeruginosa	PAO1	287	No possible amplification*
Respiratory syncytial virus	Type A (RSV-A)	208893	No match
Respiratory syncytial virus	Type B (RSV-B)	208895	No match
Rhinovirus	Type A	147711	No match
Rhinovirus	Type B	147712	No match
SARS-coronavirus	Tor2	694009	No possible amplification*
Staphylococcus epidermidis	n/a	1282	No match
Streptococcus pyogenes	n/a	1314	No possible amplification
Streptococcus salivarius	ATCC® BAA-1024D-5 CCHSS3	1304	No possible amplification
Streptococcus pneumoniae	ATCC 700669 NCTC11032	1313	No match

* Sequence match with one of the primers/probes showed <80% homology.

† Sequence match with one of the primers/probes showed ≥80% homology.

In vitro analysis

The cross-reactivity was verified in vitro with pathogens showing ≥ 80% homology with the SARS-CoV-2 Amp Primers in the in silico analysis. Samples were prepared by spiking potential

cross-reactive organisms into nasopharyngeal swab matrix at 106 cp/mL, except for SARS CoV-1 which was tested undiluted according to its supplier's recommendation. None of these pathogens showed in vitro cross-reactivity.

The microbial interference of the artus SARS-CoV-2 Prep&Amp UM Kit assay has been assessed in vitro on a panel of recommended pathogens (Table 16). Samples were prepared by spiking a maximum of 5 pathogens - at 105 TCID50/mL for viral targets, 106 cp/mL for bacterial and fungal targets, or at the highest concentration possible based on the stock concentration - into negative nasopharyngeal swabs spiked at 2.87 x LoD with inactivated SARS-CoV-2 particles (Zeptometrix). The NATtrol™ Panels and the SARS-CoV-1 were spiked directly with inactivated SARS-CoV-2 viral particles (Zeptometrix) at 2.87 x LoD. The results for each tested microorganism pools and the respective concentrations are summarized below.

Table 16 shows the list of tested pathogens in microbial interference.

Table 16. List of in vitro tested pathogens in microbial interference

Pool/ Sampled ID	Microorganism	Source	Final Concentration	Unit	Result
Pool 1	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.72E+03	cp/mL	No interference
	Human coronavirus 229E	Zeptometrix (0810229CFHI)	1.43E+05	TCID50/mL	
	Human coronavirus OC43	Zeptometrix (0810024CFHI)	5.86E+04	TCID50/mL	
	Human coronavirus NL63	Zeptometrix (0810228CFHI)	2.84E+04	TCID50/mL	
	Adenovirus T3	Zeptometrix (0810016CFHI)	1.43E+05	TCID50/mL	
	Parainfluenza virus 1	Zeptometrix (0810014CFHI)	9.14E+06	TCID50/mL	
	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.72E+03	cp/mL	
Pool 2	Adenovirus T31	Zeptometrix (0810073CFHI)	1.67E+04	TCID50/mL	No interference
	Parainfluenza virus 2	Zeptometrix (0810015CFHI)	4.29E+04	TCID50/mL	
	Influenza B Florida/02/2006	Zeptometrix (0810037CFHI)	1.43E+05	TCID50/mL	
	Rhinovirus T 1A	Zeptometrix (0810012CFNHI)	2.86E+04	TCID50/mL	

Table 16. List of in vitro tested pathogens in microbial interference (continued)

Pool/ Sampled ID	Microorganism	Source	Final Concentration	Unit	Result
Pool 3	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.72E+03	cp/mL	No interference
	Parainfluenza Virus T3	Zeptometrix (0810016CFHI)	1.43E+07	TCID50/mL	
	Haemophilus influenzae	ATCC (51907D-5)	1.00E+06	CFU/mL	
	Streptococcus pneumoniae	ATCC (700669DQ)	3.30E+06	CFU/mL	
	Candida albicans	Zeptometrix (0801504DNA)	1.00E+06	CFU/mL	
Pool 4	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.73E+03	cp/mL	No interference
	Adenovirus T7A	Zeptometrix (0810021CFHI)	1.02E+06	TCID50/mL	
	Streptococcus pyogenes	ATCC (700294DQ)	1.00E+07	CFU/mL	
	Mycoplasma pneumoniae	Zeptometrix (0801579DNA)	1.00E+08	CFU/mL	
	Pseudomonas aeruginosa	ATCC (47085DQ)	1.00E+07	CFU/mL	

Table 16. List of in vitro tested pathogens in microbial interference (continued)

Pool/ Sampled ID	Microorganism	Source	Final Concentration	Unit	Result
Pool 5	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.72E+03	cp/mL	No interference
	Respiratory syncytial virus RSVA	Zeptometrix (0810482CFHI)	7.14E+04	TCID50/mL	
	Influenza A H1N1 California	Zeptometrix (0810165CFHI)	1.43E+04	TCID50/mL	
	Enterovirus Type 68 Major Group	Zeptometrix (0810300CFHI)	1.43E+05	TCID50/mL	
	Adenovirus T14	Zeptometrix (0810108CFHI)	2.86E+04	TCID50/mL	
Pool 6	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.73E+03	cp/mL	No interference
	MERS-coronavirus	Zeptometrix (0810575CFHI)	1.43E+04	TCID50/mL	
	AdenoVirus T4	Zeptometrix (0810070CFHI)	1.43E+05	TCID50/mL	
	Human Metapneumovirus (hMPV) Type B	Zeptometrix (0810156CFHI)	7.14E+03	TCID50/mL	
	Respiratory Syncytial Virus Type B (RSV-B)	Zeptometrix (0810040CFHI)	1.43E+03	TCID50/mL	

Table 16. List of in vitro tested pathogens in microbial interference (continued)

Pool/ Sampled ID	Microorganism	Source	Final Concentration	Unit	Result
Pool 7	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.73E+03	cp/mL	No interference
	Adenovirus T5	Zeptometrix (0810020CFHI)	6.43E+05	TCID50/mL	
	Parainfluenza virus 4B	Zeptometrix (0810060BCFHI)	7.14E+04	TCID50/mL	
	Influenza A H3N2 Switzerland/9715293/13	Zeptometrix (0810511CFHI)	2.86E+04	TCID50/mL	
	Streptococcus salivarius	Zeptometrix (BAA-1024D-5)	1.00E+06	CFU/mL	
Pool 8	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.73E+03	cp/mL	No interference
	NATrol Panel RP1 (Influenza A H3N2 (Brisbane/10/07), Influenza A H1N1 (NY/02/2009), Rhinovirus (Type 1A), Adenovirus T3, Parainfluenza T1, Parainfluenzavirus T4, Metapneumovirus (Peru 6-2003) C. pneumoniae (CWL-029), M. pneumoniae (M129), Coxsackievirus (Type A1)	Zeptometrix (MDZ001)	Unknown*	N/A	

Table 16. List of in vitro tested pathogens in microbial interference (continued)

Pool/ Sampled ID	Microorganism	Source	Final Concentration	Unit	Result
Pool 9	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.73E+03	cp/mL	No Interference
	NATrol Panel RP2 (Influenza A H1 (New Caledonia/20/99), Influenza B (Florida/02/06), RSV-A, Parainfluenza T2, Parainfluenza T3, Coronavirus HKU recombinant, Coronaviruses (OC43, NL63, 229E), Bordetella pertussis (A639)	Zeptometrix (MDZ001)	Unknown*	N/A	
Pool 10	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.73E+03	cp/mL	No interference
	SARS-CoV-1	Zeptometrix (NATSARS-ST)	Unknown*	N/A	

Interfering substances

Nasopharyngeal swab samples

The effect of putative interfering substances (for the substances listed in Table 17) has been assessed on the artus SARS-CoV-2 Prep&Amp UM Kit performance. Tests were performed in 3 pools of negative nasopharyngeal swabs and in 3 pools of positive nasopharyngeal swabs spiked at 4 x LoD with inactivated SARS-CoV-2 viral particles (Zeptometrix). The experiments were performed on the RGQ MDx 5plex HRM platform (across 4 instruments) by 1 operator with 1 pilot kit.

Each pool was split into 2 to test either the interfering substance dissolved in a solvent (test sample) or the solvent alone (control sample). Hit rates in the Green and in the Red fluorescence channels were compared between the test and its corresponding control samples. In absence of interference, the test and its corresponding control samples have the same hit rate.

Table 17 shows that none of the tested substances interfere with the artus SARS-CoV-2 Prep&Amp UM Kit performance in the Green fluorescence channel.

Table 17. List of interfering substances and the hit rates obtained in Green channel

Interfering substances	Function	Tested concentration	Hit Rate results in negative nasopharyngeal swab	Hit rate results in positive (4x LoD) nasopharyngeal swab
Tobramycin	Systemic antibiotic	1 mg/mL	No interference 0/15	No interference 15/15
Mupirocin	Nasal antibiotic ointment	6.6 mg/mL	No interference 0/15	No interference 15/15
Fluticasone	Nasal corticosteroid	5% (v/v)	No interference 0/15	No interference 15/15
Menthol (Throat lozenges)	Oral anesthetic and analgesic	0.5 mg/mL	No interference 0/15	No interference 15/15
Oxymetazoline	Nasal spray	10% (v/v)	No interference 0/15	No interference 15/15
Oseltamivir	Anti-viral drug	3.3 mg/mL	No interference 0/15	No interference 15/15
Mucin (Bovine submaxillary gland type I-S)		2.5 mg/mL	No interference 0/15	No interference 15/15
Whole Blood		4% (v/v)	No interference 1/15*	No interference 15/15

*An amplification corresponding to an artefact has been detected.

Sample stability study

The samples stability study was performed to assess the impact of different sample storage conditions on the qualitative (hit rate analysis) and quantitative (Ct drift analysis) results of the artus SARS-CoV-2 Prep&Amp UM kits. Experiments were performed by analyzing two dilution levels: (1) negative samples and (2) contrived positive samples obtained by spiking of inactivated SARS-CoV-2 viral particles (Zeptometrix). To confirm the stability of the NPS samples, it was required that ≥95% of the replicates give the same hit rate and a Ct drift ≤ 10% than the time point 0 for each stability condition occurs.

Nasopharyngeal samples:

The different stability conditions tested are listed in the Table 18. Tests were performed using 3 sample pools. Negative NPS samples, 5x LoD (4750 cp/mL) of contrived positive NPS samples, and three lots of batch release samples BRS1 (N2 string, 1000 cp/10µL), BRS2 (RNAse P gblock, 1000 cp/10µL), and BRS3 (N1 string, 1000 cp/10µL) were tested with the ABI 7500 Fast Dx platform.

From the qualitative and the quantitative analysis results, the samples storage conditions tested did not impact the hit rate (the same status detected as expected) and did not lead to significant Ct drifts of the results of the artus SARS-CoV-2 Prep&Amp UM kit. Thus, performance of the kit was stable despite all different storage conditions of NPS samples tested (see Table 18).

Table 18 shows the nasopharyngeal samples stability conditions.

Table 18. Nasopharyngeal sample stability conditions

Conditions	Sample stability claim
F/T	3 F/T

Table 18. Nasopharyngeal sample stability conditions (continued)

Conditions	Sample stability claim
4°C (2°C to 8°C)	72 h
–70°C	2 weeks

Precision

The Precision study assessed the reproducibility (the same sample is repeated in different runs and conditions: 5 days, 3 kit lots, 3 operators, and 2 instruments) and the repeatability (the same sample is repeated in the same run and condition). Tests were performed on negative nasopharyngeal samples and negative nasopharyngeal samples spiked at 5 x LoD on the RGQ MDx.

For each dilution level, 204 data points were collected. Repeatability and reproducibility data were used to determine the standard deviation (SD) and the coefficient of variation (%CV) for the SARS-CoV-2 targets in the Green, Yellow, and Red channels. Table 19 shows that the artus SARS-CoV-2 Prep&Amp UM Kit has an overall precision of 0.63 SD (2.03% CV) in the Green channel, 0.54 SD (2.22 %CV) in the Yellow channel, and of 1.28 SD (4.10 %CV) in the Red channel.

Table 19. Standard deviation and coefficient of variation of the artus SARS-CoV-2 Prep&Amp UM Kit

Samples and detection channel	Total	Day-to-day	Batch-to-batch	Operator-to-operator	Instrument-to-instrument	Run-to-run	Within run
	Standard deviation (SD) (Coefficient of variation (%CV))						
Negative NPS Yellow channel	0.54 (2.22)	0.09 (0.37)	0.10 (0.42)	0.06 (0.27)	0.11 (0.47)	0.09 (0.36)	0.50 (2.05)
Negative NPS Red channel	1.15 (3.68)	0.0 (0.00)0	0.55 (1.76)	0.00 (0.00)	0.12 (0.40)	0.39 (1.26)	0.92 (2.96)

Table 19. Standard deviation and coefficient of variation of the artus SARS-CoV-2 Prep&Amp UM Kit (continued)

Samples and detection channel	Total	Day-to-day	Batch-to-batch	Operator-to-operator	Instrument-to-instrument	Run-to-run	Within run
	Standard deviation (SD) (Coefficient of variation (%CV))						
Spiked NPS Green channel	0.63 (2.03)	0.18 (0.59)	0.31 (1.00)	0.00 (0.00)	0.08 (0.25)	0.00 (0.00)	0.51 (1.64)
Spiked NPS Yellow channel	0.47 (1.93)	0.13 (0.53)	0.24 (0.98)	0.05 (0.20)	0.18 (0.73)	0.00 (0.00)	0.33 (1.38)
Spiked NPS Red channel	1.28 (4.10)	0.12 (0.37)	0.58 (1.84)	0.11 (0.34)	0.00 (0.00)	0.49 (1.57)	1.02 (3.27)

Clinical performance

Nasopharyngeal swabs including asymptomatic individuals

The clinical performance of the *artus* SARS-CoV-2 UM Prep&Amp assay was evaluated using retrospective nasopharyngeal swab specimens in transport medium, consisting of 694 clinical specimens.

All specimens were collected from patients with or without symptoms or other reasons to suspect COVID-19 infection.

The clinical validation was performed on the RGQ MDx. Three samples (0.4%) were excluded from analysis after testing with the *artus* SARS-CoV-2 Prep&Amp UM Kit due to an invalid status according to the sample validity criteria (Table 20).

Two reference methods were used depending on the source of the sample (Perkin-Elmer® and the Roche cobas® SARS-CoV-2 assay).

Table 20 reports the performance of the *artus* SARS-CoV-2 Prep&Amp UM Kit against a reference method, which is expressed as positive percent agreement (PPA) and negative percent agreement (NPA).

Table 20. Clinical performance of the artus SARS-CoV-2 Prep&Amp UM Kit against a reference method

Sample Status	N	% of Positive	95% CI	% Negative	95% CI
Positive	122	91.80 (112/122)	85.44 – 96.00	8.20 (10/122)	–
Negative	569	1.58 (9/569)	–	98.42 (560/569)	97.0 2– 99.27

Nineteen discordant results were evaluated by a third method and re-analyzed with a contingency table. The overall clinical performance results are expressed as positive percent agreement (PPA) and negative percent agreement (NPA) and are shown in Table 21.

Table 21. Clinical performance of the artus SARS-CoV-2 Prep&Amp UM Kit after discordant results analysis

Sample Status	N	% of Positive	95% CI	% Negative	95% CI
Positive	123	91.87 (113/123)	85.56 – 96.03	8.13 (10/123)	–
Negative	568	1.41 (8/568)	–	98.59 (560/568)	97.24 – 99.39

Ten false negative samples by the candidate method were called as positive by the discordant resolution method and remained as false negatives in the post discordant assessment. Of the nine false positive results by the candidate method, eight were called as negative by the discordant resolution method and remained as false positives in the post discordant assessment. One false positive result was called as positive by the discordant resolution method and so was treated as a true positive in the post discordant assessment.

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Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support** (for contact information, visit **www.qiagen.com).**

	Comments and suggestions
Weak or No Green signal (FAM) in Positive Control (PC)	
a) The selected fluorescence channel for RT-PCR data analysis does not comply with the protocol.	For data analysis, select the fluorescence channel FAM (Green) for the analytical SARS-CoV-2 RT-PCR targets, the fluorescence channel HEX/VIC/JOE (Yellow) for the sampling control and the Cy5/Atto (Red) for the internal control.
b) Incorrect programming of the temperature profile.	Compare the RT-PCR program with the protocol.
c) Incorrect configuration of the PCR reaction	Verify your work steps through the pipetting scheme and repeat the PCR, if necessary.
d) The storage conditions for one or more kit components did not comply with the instructions, or the artus SARS-CoV-2 RT-PCR kit has expired.	Follow the storage conditions and verify the reagents' expiration date and use a new kit, if necessary.
e) Incorrect configuration of the real time RT-PCR platform during the data configuration.	Apply the recommended configurations related to your real time RT-PCR platform that are described in this manual.
f) The PCR was inhibited.	Follow the good practices in molecular biology laboratory to avoid the introduction of contaminants. Make sure that workspace and instruments are decontaminated at regular intervals. Follow the protocol mentioned in this manual. Check the expiration date of the reagent and use a new kit, if necessary. Repeat the assay with another sample.
Green signal (FAM) in the No Template Control or in the No Extraction Control	

	Comments and suggestions
Contamination with SARS-CoV-2 sequences occurred during the RT-PCR plate preparation.	Repeat the RT-PCR with new reagents. Follow the good practices in molecular biology laboratory to avoid the introduction of contaminants. Follow the protocol mentioned in this handbook. Make sure that workspace and instruments are decontaminated at regular intervals.
Weak or no Red signal (Cy5/Atto) from the Internal control	
a) An interferent has been introduced in the RT-PCR reaction. The PCR is inhibited.	Follow the good practices in molecular biology laboratory to avoid the introduction of contaminants. Make sure that workspace and instruments are decontaminated at regular intervals. Follow the protocol mentioned in this manual. Repeat the experiment with a newly collected sample.
b) The internal control is degraded.	Follow the good practices in molecular biology laboratory to avoid the introduction of RNases. Follow the recommendations mentioned in this manual. Make sure that workspace and instruments are decontaminated at regular intervals. Follow the storage conditions and check the reagents' expiration date and use a new kit, if necessary.
c) Incorrect configuration of the real time RT-PCR platform during the data configuration.	Apply the recommended configurations related to your real time RT-PCR platform that are described in this manual.
Weak or no Yellow signal (VIC/HEX) of the sampling control	
a) The clinical sample is degraded.	Follow the recommendations provided by the collection device supplier for their storage, handling and transport. Follow the protocol mentioned in this manual, including the sample preparation steps with the SARS-CoV-2 UM Prep buffer. Follow the storage conditions and check the reagents' expiration date, such as the SARS-CoV-2 UM Prep buffer, and use a new kit, if necessary.
b) The specimen was not properly collected. Not enough human cells were collected on the swab or transferred in the transport media.	Follow the recommendations provided by the collection device supplier for the specimen collection and the specimen handling.
c) Incorrect configuration of the real-time RT-PCR platform during the data configuration.	Apply the configurations related to your real time RT-PCR platform that are described in this manual.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labelling:

Symbol	Symbol definition
	Contains reagents sufficient for <N> reactions
	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
GTIN	Global Trade Item Number
CONT	Contains
COMP	Component
NUM	Number
Rn	R is for revision of the Instructions for Use and n is the revision number

Symbol	Symbol definition
	Temperature limitation
	Manufacturer
	Consult instructions for use downloadable from **www.qiagen.com**
	Protect from light
	Warning/Caution

Contact Information

For technical assistance and more information, please contact the QIAGEN Technical Services at **support.qiagen.com**.

Ordering Information

Product	Contents	Cat. no.
artus SARS-CoV-2 Prep&Amp UM Kit (768)	For 768 reactions: Preparation Buffer, ROX dye, Master Mix, Primers and Probes, Internal Control, Water (NTC), and Positive Control	4511460
artus SARS-CoV-2 Prep&Amp UM Kit (3072)	For 3072 reactions: Preparation Buffer, ROX dye, Master Mix, Primers and Probes, Internal Control, Water (NTC), and Positive Control	4511469
Instrument and accessories		
PCR tubes, 0.1 ml for Rotor-Gene Q 5-plex HRM MDx	For use with 72-well rotor, Strip tubes, and caps	981103
Rotor-Gene Q software	Rotor-Gene Q software v2.3.1 (or higher)	
Rotor-Gene Q MDx 5-plex HRM Platform	Real-time PCR cycler with 5 channels, high-resolution melt analyzer, software, laptop computer, and accessories; 1-year warranty on parts and labor, installation	9002032
72-Well Rotor	For holding Strip Tubes and Caps, 0.1 ml, with reaction volumes of 10–50 µl	9018903
Locking Ring 72-Well Rotor	For locking Strip Tubes and Caps, 0.1 ml in the 72-Well Rotor	9018904

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit Instructions for Use. QIAGEN kit Instructions for Use are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Revision	Description
R1, January 2024	Initial release

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