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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 Only. Validation of this test has not been reviewed by the FDA. Review under the EUA program is pending. The test is distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.

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For Prescription Use Only

For use on Rotor-Gene® Q MDx and ABI® 7500 Fast Dx instruments

REF 4511440, 4511449

QIAGEN GmbH
QIAGEN Strasse 1, 40724 Hilden, GERMANY

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Sample to Insight

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Contents

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 the SARS-CoV-2 in nasopharyngeal swabs (NPS), nasal swabs, and oropharyngeal swabs from individuals with signs and symptoms of infection who are suspected of COVID-19. 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 or by similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in NPS, nasal swabs, and oropharyngeal swabs during the acute phase of infection. Positive results are indicative of active infection. 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 *artus* SARS-CoV-2 Prep&Amp UM Kit 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. Validation of this test has not been reviewed by the FDA. Review under the EUA program is pending. The test is distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.

The *artus* SARS-CoV-2 PCR assay test is intended to be used with the Rotor-Gene Q (RGQ) MDx System or the ABI 7500 Fast Dx as RT-PCR instruments.

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 respiratory disease in Wuhan City in China (1, 3). First named novel coronavirus (2019-nCoV), SARS-CoV-2 differs from the SARS-CoV (1, 3), which was responsible for the 2003 outbreak, and the MERS-CoV, which has been circulating in the Middle East since 2012. SARS-CoV-2 is the causative agent of COVID-19. The SARS-CoV-2 RNA is detectable during the early and acute phases of the infection from various upper respiratory tract specimens (nasal, oropharyngeal, and nasopharyngeal swabs) (3).

The SARS-CoV-2 Prep&Amp UM assay targets 2 viral genes (N1 and N2 genes) detected with the same fluorescence channel. The two gene targets are not differentiated, and amplification of either or both gene targets leads to a fluorescence signal. Positive results are indicative of the presence of the SARS-CoV-2 virus, but do not rule out co-infection with other pathogens. On the other hand, negative 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 RT-PCR on either the RGQ MDx system or on ABI 7500 Fast Dx platform (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 regions of the SARS-CoV-2 RNA genome and for their direct detection in the "Green" fluorescence channel of the RGQ MDx instruments and with the fluorescent filter A/1 of the ABI 7500 Fast Dx.

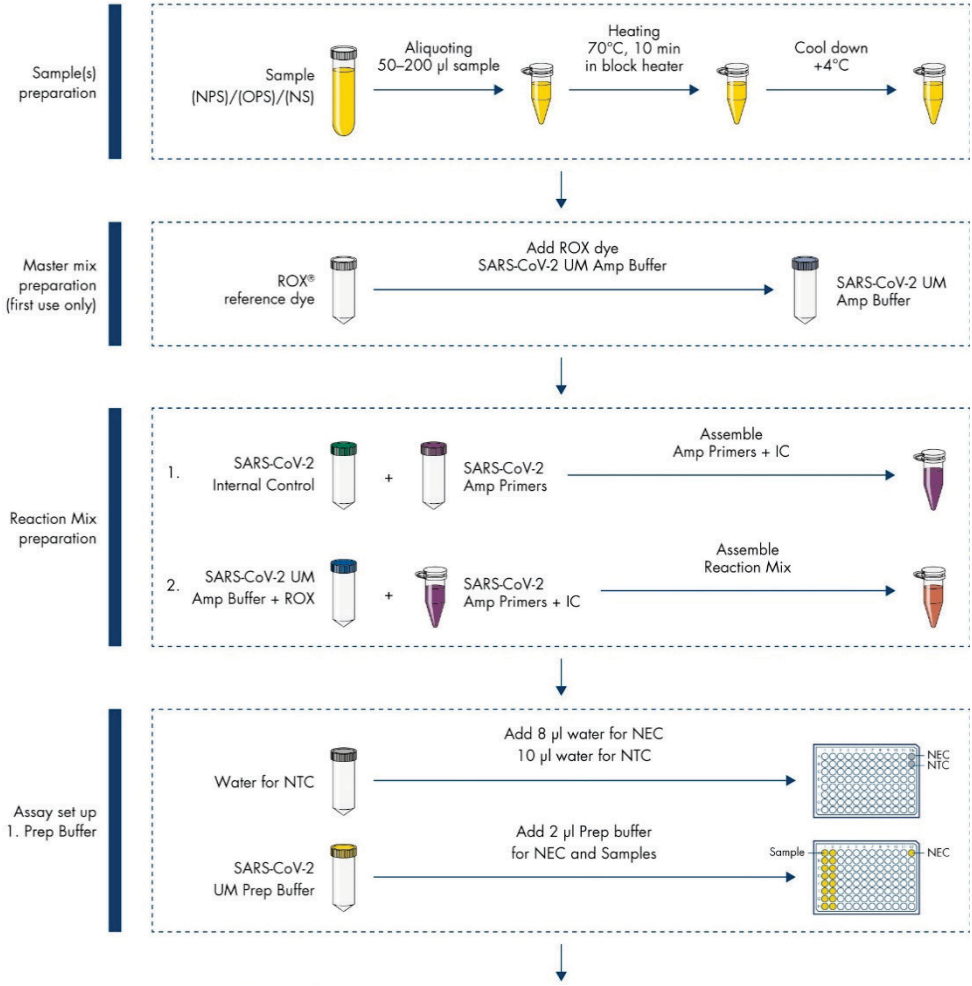
The Primers and Probes mix of the *artus* SARS-CoV-2 Prep&Amp UM Kit also contains the oligonucleotides required for the RNAse P amplifications. When detected in the "Yellow" fluorescence channel of the RGQ MDx instrument or with the fluorescent filter B/2 of the ABI 7500 Fast Dx, those amplicons assure that enough biological sample has been collected on the swab. 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 RT-PCR inhibition. This is detected as an internal RNA control (IC) in the "Red" fluorescence channel of the RGQ MDx instruments and with the fluorescence filter E/5 of the ABI 7500 Fast Dx. Because the IC is included in the SARS–CoV–2 Amp Primers Mix, its amplification should be constant, unless an RT-PCR inhibitor is present in the sample or in the RT-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 RT-PCR inhibitors in the preparation buffer.

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

artus SARS-CoV-2 Prep&Amp UM Kit Workflow



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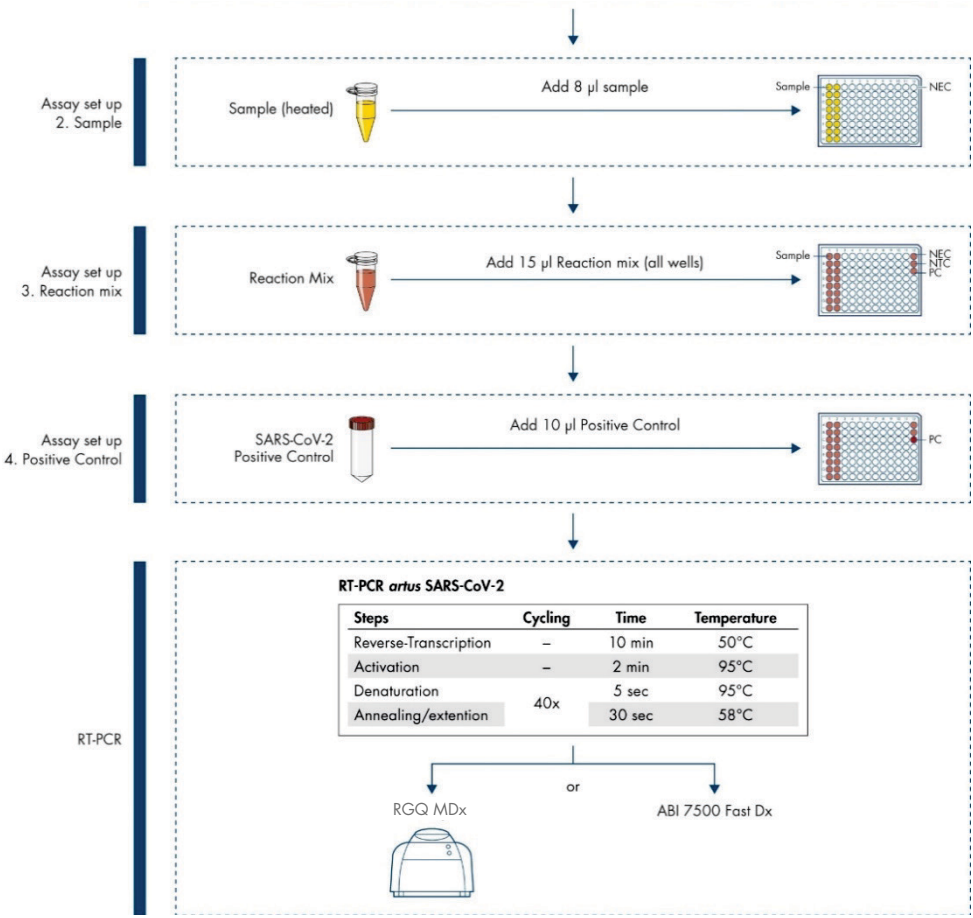

Figure 1. *artus* SARS-CoV-2 Prep&Amp UM Kit workflow.

Materials Provided

Kit contents

***artus* SARS-CoV-2 Prep&Amp UM Kit** **Catalog no.** **Number of reactions**				**4511440** **768**	**4511449** **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 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 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 step (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 two workflows that require the use of the Rotor-Gene Q MDx or of the ABI 7500 Fast Dx instruments and their appropriate software:

- Rotor-Gene Q MDx: 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

Consumables

- Disposable powder-free gloves
- Sterile and nuclease-free pipette tips with filters
- 1.5 ml or 2 ml PCR-free tubes
- 0.1 ml PCR tubes for use with the Rotor-Gene Q MDx (Strip Tubes and Caps, 0.1 ml, cat. no. 981103)
- 96-Well MicroAmp™ for use with the ABI 7500 Fast Dx qPCR platform (Applied Biosystems 96-well plate, cat. no. N8010560)
- MicroAmp Optical Adhesive film for use with the ABI 7500 Fast Dx qPCR platform (Applied Biosystems, cat. no. 4360954)

Equipment*

- Desktop centrifuge with rotor for 2 ml reaction tubes
- Pipettes (adjustable)
- Vortex mixer
- Block heater
- Rotor-Gene Q MDx (cat. no. 9002035 or 9002036) with Rotor-Gene Q software version 2.3.1 or higher
- Rotor-Disc 72 Rotor (cat. no. 9018899)
- Rotor-Disc 72 Locking Ring (cat.no. 9018900)
- 72-well Loading Block (loading block 72 x 0.1 ml tubes, cat. no 9018901)
- Alternatively: ABI 7500 Fast Dx qPCR platform (Thermo Fisher Scientific, cat. no 4406985) with software version 1.4.1 or higher and a 96-well plate centrifuge

* Prior to use and when applicable, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

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

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 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 all components' labels. 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 for 6 months, or until expiry date.

Specimen Transport, Storage and Handling

The *artus* SARS-CoV-2 Prep&Amp UM Kit is for use with nasopharyngeal, nasal, and oropharyngeal 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.

Specimen collection, transport and storage

For swab specimen collection, storage, and transport, please refer to the supplier's recommendations. Swabs must be fully immersed in transport media to maintain specimen integrity.

Protocol: Sample preparation and SARS-CoV-2 detection on the RGQ MDx

This protocol describes the sample and the RT-PCR preparation to detect the SARS-CoV-2 targets in human nasal, nasopharyngeal, or oropharyngeal swabs stored in transport media on the RGQ MDx.

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

- Samples may be kept at room temperature 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 (15–25°C). 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 qPCR 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

1. Sample preparation
   1a. Vortex the swab containing the sample vigorously.
   1b. Aliquot 50-200 µl of the sample into 1.5mL PCR-free tubes
   1c. 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.
   2a. Add 32.8 µl of the ROX dye to 1 tube of SARS-CoV-2 UM Amp Buffer.
   2b. Close the lid containing the SARS-CoV-2 UM Amp Buffer and the ROX dye and invert the tube 3 times.
   2c. 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.
   3a. 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.
   3b. Close the lid and invert the tube 3 times or pulse vortex the tube for 3-5 s.
   3c. 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 (+22% extra volume*)**
SARS-CoV-2 Amp Primers	**3.45x**	1x	7.25	638
SARS-CoV-2 Internal Control	166.67 cp/µl	10 cp/µl	1.5	132
Total SARS-CoV-2 Amp Primers + IC mix			8.75	770

* **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.

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†	4x	1x	6.25	540
SARS-CoV-2 Amp Primers‡	**2.9x**	1x	8.75	756
Total reaction volume		–	15.00	1296

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

† SARS-CoV-2 UM Amp buffer completed with the ROX Reference Dye

‡ SARS-CoV-2 Amp Primers completed with the SARS-CoV-2 Internal Control

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 4 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 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	5 s	95	40	No
Annealing/Extension	30 s	58		Green (FAM), Yellow (HEX), and Red (Atto)

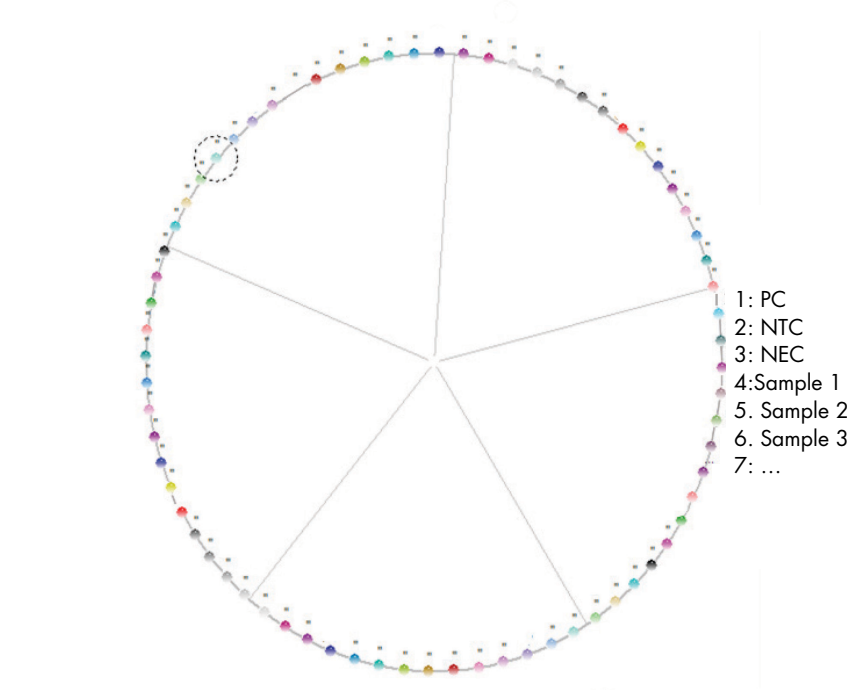

Figure 2. Example of tube layout on the RGQ MDx platform

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 configuration

Name	PC tube position	Min reading (FI)	Max reading (FI)	Min gain	Max gain
Green	1*	5	10	–10	10
Yellow	1*	5	10	–10	10
Red	1*	5	10	–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 the 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 nasal, nasopharyngeal, or oropharyngeal swabs stored in transport media on the ABI 7500 Fast Dx qPCR 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

- Samples may be kept at room temperature 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 (15–25°C). 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 is not a virus-inactivating solution. All samples should be treated as potentially hazardous.
- Verify that the cycling conditions of the qPCR 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

1. Sample preparation
   1a. Vortex the swab containing sample vigorously.
   1b. Aliquot 50-200 µl of sample into 1.5mL PCR-free tubes.
   1c. Perform heating step at 70°C for 10 min on a block heater. 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.
   2a. Add 32.8 µl of the ROX dye to a tube of SARS-CoV-2 UM Amp Buffer.
   2b. Close the lid containing the SARS-CoV-2 UM Amp Buffer and the ROX Dye and invert the tube 3 times.
   2c. 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.
   3a. 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.
   3b. Close the lid and invert the tube 3 times or pulse vortex the tube for 3-5 s.
   3c. 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 (+ 21% extra volume*)**
SARS-CoV-2 Amp Primers	**3.45x**	1x	7.25	841
SARS-CoV-2 Internal Control	166.67 cp/µl	10 cp/µl	1.5	174
Total SARS-CoV-2 Amp Primers + IC mix			8.75	1015

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

4. Prepare a reaction mix according to Table 6 and mix thoroughly.

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†	4x	1x	6.25	720
SARS-CoV-2 Amp Primers‡	**2.9x**	1x	8.75	1008
Total reaction volume	–		15.00	1728

***** **Note**: Adjust the volumes 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.

† SARS-CoV-2 UM Amp Buffer completed with the ROX Reference Dye

‡ SARS-CoV-2 Amp Primers completed with the SARS-CoV-2 Internal Control

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 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 4 to the wells dedicated to samples and controls (Figure 3 provided as an example). 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 RT-PCR program on the "Standard 7500" Run Mode of the ABI 7500 Fast Dx according to Table 7.

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

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

14. Place the plate in the real-time cycler (an example of a PCR plate layout is represented in the 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 the 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	5 s	95	40	No
Annealing/Extension	30 s	58		Green (FAM), Yellow (VIC), and Red (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

On the RGQ MDx, 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 6, September 2018). The following analysis parameters are needed for consistency between different analyses (Table 8).

Table 8. Analysis parameters for the RGQ MDx

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	No
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 from selected samples are summarized in the table and can be exported as an Excel® file by right-clicking the mouse button in the grid and selecting **Export to Excel**. Make sure that all samples are selected before exporting the results.

On the ABI, the data are analyzed with the 7500 Fast System Software version 1.4.1 (or higher) according to the manufacturer's instructions. The following parameters are needed for consistency between different analyses (Table 9).

Table 9. Analysis parameters for the ABI 7500 Fast Dx

Channels	FAM*	VIC/HEX*	CY5/Atto*
Passive dye	ROX	ROX	ROX
Fluorescence threshold	0.13	0.05	0.025
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

* FAM = Filter A/1 in ABI platform, VIC/HEX = Filter B/2 in ABI platform, Cy5/Atto = Filter E/5 in ABI platform

In the ABI SDS software, Ct values of a selected group of wells or the entire plate are available in the **Report** sheet of the **Results** main section. Data can be exported in comma separated value text (.csv) format (recommended): 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 (or in the fluorescent channel FAM on the ABI).

The sampling control, composed of the RNAse P, is detected in the Yellow fluorescence channel with the RGQ MDx (or in the fluorescence channel VIC/HEX with the ABI). 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 a 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 (or in the fluorescence channel Cy5/Atto with the ABI).

To validate the RT-PCR runs, the PC, the NTC, and the NEC controls must be amplified and detected as expected.

Table 10. Run validity criteria and result interpretation on the RGQ MDx.

Control	Detection in green channel	Detection in Yellow channel	Detection in Red channel	Interpretation
Positive control (PC)	Ct ≤ 38.00	Indifferent	Indifferent	Run is validated.
	Ct > 38.00 or No Ct	Indifferent	Indifferent	Run is invalidated.
No template control (NTC) or No extraction control (NEC)	Ct > 38.00 or No Ct	Ct > 35.00 or No Ct	Yes	Run is validated.
	Any other combinations with amplification in green or yellow		Indifferent	Run is invalidated.

Table 11. Run validity criteria and result interpretation on the ABI 7500 Fast Dx

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

* FAM = Filter A/1 in ABI platform, VIC/HEX = Filter B/2 in ABI platform, Cy5/Atto = Filter E/5 in ABI platform

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

Table 12. Sample validity criteria and results interpretation on the RGQ MDx.

Detection in Green channel	Detection in Yellow channel	Detection in Red channel	Interpretation
Ct ≤ 38	Indifferent	Indifferent	Sample is positive for SARS-CoV-2 RNA.
Ct > 38 or No Ct	Ct ≤ 35.00	Indifferent	Sample is negative, SARS-CoV-2 RNA is not detected.
Ct > 38 or No Ct	Ct > 35.00 or No Ct	Yes	Invalid sample. No or insufficient human material detected. Re-sampling is required.
Ct > 38 or No Ct	Ct > 35.00 or No Ct	**No**	Invalid sample. RT-qPCR reaction is inhibited. A retest is required.

Table 13. Sample validity criteria and results interpretation on the ABI 7500 Fast Dx.

Detection in FAM dye	Detection in VIC/HEX dye	Detection in Cy5/Atto dye	Interpretation
Ct ≤ 39	Indifferent	Indifferent	Sample is positive for SARS-CoV-2 RNA.
Ct > 39 or No Ct	Ct ≤ 35.00	Indifferent	Sample is negative, SARS-CoV-2 RNA is not detected.
Ct > 39 or No Ct	Ct > 35.00 or No Ct	Yes	Invalid sample. No or insufficient human material detected. Re-sampling is required.
Ct > 39 or No Ct	Ct > 35.00 or No Ct	No	Invalid sample. RT-qPCR reaction is inhibited. A retest is required.

* FAM = Filter A/1 in ABI platform, VIC/HEX = Filter B/2 in ABI platform, Cy5/Atto = Filter E/5 in ABI platform

Limitations

- For *in vitro* diagnostic use only. Validation of this test has not been reviewed by FDA. Review under the EUA program is pending. The test is distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.
- A statement such as "The test has been validated, but FDA's independent review of this validation is pending" should be included in test reports to healthcare providers.
- 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 specially instructed and trained in the *in vitro* diagnostics procedures.
- Strict compliance with the qPCR platform's user manual (Rotor-Gene Q 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.
- The performance of this test has not been established for patients without signs and symptoms of respiratory infection.

Performance

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®). 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 confirmed on both claimed real-time PCR platforms using two different lots of reagents.

The claimed limit of detection for both real-time PCR platforms for the *artus* SARS-CoV-2 Prep&Amp UM Kit is 950 cp/ml.

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 23/03/202) 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 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 14. 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

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

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Table 14 (Continued from previous page)

Pathogens	Strain/Type	Taxonomy ID	*In silico* PCR results
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
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 shoved ≥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 10^6 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. Samples were prepared by spiking a maximum of 5 pathogens - at 10^5 TCID50/mL for viral targets, 10^6 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 15. List of *in vitro* tested pathogens in microbial interference.

Pool ID / Sample 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	
Pool 2	SARS-CoV-2	Zeptometrix (NATSARS(COV2)-ERC)	2.72E+03	cp/ml	No interference
	Adenovirus T31	Zeptometrix (0810073CFHI)	1.67E+04	TCID50/ml	
	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	

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Table 15 (Continued from previous page)

Pool ID / Sample 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	
	Staphylococcus epidermidis	ATCC (12228DQ)	4.60E+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	
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	

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Table 15 (Continued from previous page)

Pool ID / Sample 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	
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	

* Concentration not communicated by the supplier.

Interfering substances

The effect of putative interfering substances (for the substances listed in the Table 16) 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 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 16 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 16. List of interfering substances.

Interfering substances	Function	Tested concentration	Results in negative nasopharyngeal swab	Results in positive (4x LoD) nasopharyngeal swab
Tobramycin	Systemic antibiotic	1 mg/ml	No interference 0/15	No interference 0/15
Mupirocin	Nasal antibiotic ointment	6.6 mg/ml	No interference 0/15	No interference 0/15
Fluticasone	Nasal corticosteroid	5% (v/v)	No interference 0/15	No interference 0/15
Menthol (Throat lozenges)	Oral anesthetic and analgesic	0.5 mg/ml	No interference 0/15	No interference 0/15

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Table 16. (Continued from previous page)

Interfering substances	Function	Tested concentration	Results in negative nasopharyngeal swab	Results in positive (4x LoD) nasopharyngeal swab
Oxymetazoline	Nasal spray	10% (v/v)	No interference 0/15	No interference 0/15
Oseltamivir	Anti-viral drug	3.3 mg/ml	No interference 0/15	No interference 0/15
Mucin (Bovine submaxillary gland type I-S)		2.5 mg/ml	No interference 0/15	No interference 0/15
Whole Blood		4% (v/v)	No interference 1/15*	No interference 0/15

* An amplification corresponding to an artefact has been detected.

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 17 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 1.28 SD (4.10 %CV) in the red channel.

Table 17. 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)
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

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

- 98 SARS-CoV-2 RNA negative specimens
- 52 SARS-CoV-2 RNA positive nasopharyngeal specimens

All specimens were collected from patients with signs and symptoms of infection who were suspected of COVID-19 and were stored frozen until use.

The clinical validation was performed on the ABI 7500 Fast Dx. Table 18 reports the performance of the *artus* SARS-CoV-2 Prep&Amp UM Kit against a n RT-PCR reference method.

Table 18. Clinical performance of the *artus* SARS-CoV-2 Prep&Amp UM Kit against a reference method

Reference method result	N	% Positive	95% CI	% Negative	95% CI
Positive	52	98.1 (51/52)	89.9 – 99.7	5.1 (5/98)	–
Negative	98	1.9 (1/52)	–	94.9 (93/98)	88.7 – 97.8

Of note, samples with discordant results were evaluated by a third method. The overall clinical performance results did not change following the discordant testing.

Listed below are the positive percent agreement (sensitivity) and negative percent agreement (specificity):

Positive Percent Agreement (PPA%): 51/52 = 98.1% (95% CI: 89.9% - 99.7%)
Negative Percent Agreement (NPA%): 93/98 = 94.9% (95% CI: 88.6% - 97.8%)

References

1. CUI J *et al.* (2019) Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol **17**, 181-192.

2. Gagneur *et al.* (2002) Infections nosocomiales à coronavirus humains chez le nouveau-né. Arch Pédiatr **9**, 61-69.

3. HU *et al.* (2020) Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol **6**:1-14.

Troubleshooting Guide

This troubleshooting guide may help solve any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**.

	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 qPCR platform during the data configuration.	Apply the recommended configurations related to your qPCR 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 the 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	
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 the workspace and instruments are decontaminated at regular intervals.

	Comments and suggestions
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 the workspace and instruments are decontaminated at regular intervals. Follow the protocol mentioned in this manual. Repeat the experiment with a sample newly collected.
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 the 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 qPCR platform during the data configuration.	Apply the recommended configurations related to your qPCR 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 qPCR platform during the data configuration.	Apply the configurations related to your qPCR platform that are described in this manual.

Symbols

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

Symbol	Symbol definition
	Contains reagents sufficient for 768 or 3072 reactions
	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
COMP	Components
CONT	Contains
NUM	Number
GTIN	Global Trade Item Number
Rn	R is for revision of the Instructions for Use, and n is the revision number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Prescription Use Only
	Keep away from sunlight
	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	4511440
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	4511449
Instrument and accessories		
PCR tubes, 0.1 ml for Rotor-Gene Q 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	Real-time PCR cycler with 5 channels, high-resolution melt analyzer, software, laptop computer, and accessories; 1-year warranty on parts and labor, installation	9002035 or 9002036
Loading Block	72 x 0.1 ml tubes	9018901

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

Document Revision History

Revision	Description
R1, April 2021	Initial release.

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