BIOMÉRIEUX

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ARGENE®
SARS-COV-2 R-GENE®

IVD

℞ only

For use under the Emergency Use Authorization (EUA) only.

Real-time detection kit SARS-CoV-2 R-GENE®

1. INTENDED USE

The SARS-COV-2 R-GENE® assay is a real-time reverse transcriptase (RT)-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all 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 SARS-COV-2 R-GENE® assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The SARS-COV-2 R-GENE® kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. SUMMARY AND EXPLANATION OF THE TEST

2.1. Explanation of the Test

SARS-COV-2 R-GENE® is a qualitative test for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in upper respiratory samples and bronchoalveolar lavage (BAL) fluid. A RNA internal control is introduced into each sample and in a negative reference sample (water) to monitor the whole extraction process (including lysis step) and control for inhibition. Amplification positive controls are also included.

SARS-COV-2 R-GENE® kit allows to perform 2 PCR assays. **PCR1** is performed as a first step for detection of SARS-CoV-2. **PCR2** is optional and may be performed on the same eluate to monitor sample quality by confirming the presence of human cells, and to detect a third highly conserved gene that is specific to Sarbecovirus.

2.2. Principles of the Procedure

SARS-COV-2 R-GENE® test if based on the automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. Several extraction and amplification instruments have been validated for use with the kit.

Nucleic acid from sample and Internal Control (**IC1**) are simultaneously extracted. The extraction principle depends on the instrument. As an example, in EMAG® and NUCLISENS® easyMAG® system, nucleic acids are released by addition of lysis buffer (containing guanidine thiocyanate, a chaotropic agent) to lyse cells, bacteria, and viruses present in the samples and denature proteins such as nucleases. The released nucleic acids bind to magnetic silica particles. Unbound substances and impurities, such as cellular debris, denatured protein and potential PCR inhibitors are removed during washing steps. Finally, nucleic acids are eluted from the magnetic silica particles at elevated temperature and recovered in a small amount of elution buffer.

Selective amplification of target nucleic acid from sample eluate is achieved by the use of target specific forward and reverse primers for the N gene and RdRp gene (that are unique to SARS-CoV-2), along with non-competitive sequence specific forward and reverse primers for **IC1** (RNA Internal Control) in **PCR1**. For **PCR2**, amplification is achieved by the use of target-specific forward and reverse primers for the E gene (highly conserved gene amongst Sarbecovirus) and the human HPRT1 gene (cell control detection) along with non-competitive sequence specific forward and reverse primers for **IC1** (RNA Internal Control).

The kit provides a ready-to-use amplification premix containing all reagents required for the PCR amplification step. Reverse transcriptase is provided separately, to be used as needed and diluted prior addition to the amplification premix reagent. Amplification is based on 5' nuclease technology, which utilizes the 5'– 3' exonuclease activity of the Taq polymerase. The Threshold Cycle (Ct) values for each target and Internal Control (**IC1**) are calculated using the software of the validated amplification instruments. Prior to analysis of individual sample results, conditions for run validation must be verified, including confirmation that the positive and negative controls produced the expected results. The differences in Ct values for the Internal Control (**IC1** between test samples and reference, SARS-CoV-2 negative control samples) is used to monitor for assay inhibition.

3. PRINCIPLE OF THE TEST

3.1. Sample Types

Sample types that are acceptable for use with this kit are nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid. The performance of the SARS-COV-2 R-GENE® assay was established using contrived nasopharyngeal swab samples. Please refer to the FDA FAQs on Diagnostic Testing for SARS-CoV-2 for additional information regarding acceptable specimen types for detection of SARS-CoV-2.

3.2. Nucleic Acid Extraction

Extraction systems validated with this kit are the following:

- EMAG® (BIOMÉRIEUX), with software version 1.1
- NucliSENS® easyMAG® (BIOMÉRIEUX), with software version 2.1
- QIASymphony SP (QIAgen), with software version 4.1
- MagNA Pure 96 (Roche), with software version 3.1

The target RNA in the sample and in the internal control (**IC1**) is extracted using one of the validated extraction systems indicated above.

3.3. Real-time Amplification and Detection

Principle

Real-time PCR is based on the amplification of specific regions of the targeted genome. Detection is performed using the 5' nuclease hydrolysis probe technique.

The SARS-COV-2 R-GENE® kit contains all necessary reagents to detect:

- **PCR1** (120 tests): Specifically the SARS-CoV-2 genome in a triplex reaction: N gene of SARS-CoV-2 at **530 nm**, RdRp gene of SARS-CoV-2 at **670 nm**, and internal control at **560 nm**.
- **PCR2** (30 tests): Generically the Sarbecovirus (SARS-CoV, SARS-CoV-2, SARS-like) genomes in a triplex reaction: E gene of Sarbecovirus at **530 nm**, cellular control at **670 nm**, and internal control at **560 nm**.

Amplified Sequences

PCR1: The primers used allow for amplification of:
- the N gene of SARS-CoV-2. The size of the amplified fragment is 148 bp.
- the RdRp gene of SARS-CoV-2. The size of the amplified fragment is 136 bp.

PCR2: The primers used allow for amplification of:
- the E gene of Sarbecovirus. The size of the amplified fragment is 148 bp.
- the HPRT1 gene (for Cell Control). The size of the amplified fragment is 108 bp.

Validated Amplification Platforms

- Applied Biosystems
  - 7500 Fast(1) with software version 2.3
  - 7500 Fast Dx(1) with software version 1.4
  - QuantStudio 5 Dx(1) with software version 1.0
- LightCycler 480 (System II) (Roche) with software version 1.5
- CFX96 (Bio-Rad) with software Maestro 1.1
- Rotor-Gene Q (Qiagen) with software 2.3

(1) Run **Fast** mode only.

3.4. Controls

3.4.1. Extraction + Inhibition Controls

3.4.1.1. Sample Extraction + Inhibition Control (IC sample)

- This control consists of the internal control **IC1** that is added to the samples, extracted, and amplified in order to check the efficacy of the extraction, and detect the presence of possible inhibitors.
- Its signal is detected at **560 nm**.

3.4.1.2. Reference Extraction + Inhibition Control (IC W0)

- This control consists of the internal control IC1 that is added to the water (**W0**), extracted, and amplified at the same time as the samples in order to obtain a reference (**IC1W0**) per extraction run.
- Its signal is detected at **560 nm**.

⇒ Comparison of Ct (Cycle Threshold) values of **IC1W0** and **IC1sample** at **560 nm** is used to evaluate the efficacy of the extraction, and to detect the presence of possible inhibitors.

3.4.2. Negative Control (IC W0)

- This is the same as described in the *REFERENCE EXTRACTION + INHIBITION CONTROL* section; it constitutes a negative control used to check for the absence of contamination during extraction and amplification.
- Its signal is detected at **530 nm** and **670 nm**.

3.4.3. Positive Control (PC1 and PC2)

- **PC1** contains transcripts that specifically anneal with the **PCR1** primers in the **R01** reaction mixture.
- **PC2** contains a mix of transcripts and plasmids that specifically anneal with the **PCR2** primers in the **R02** reaction mixture.
- The positive control is systematically tested and ensures the amplification step is carried out properly.
- Its signal is detected at **530 nm** and **670 nm**.

3.4.4. Cell Control (Cc)

- The Cell control (Cc) checks for the presence of cells in the sample, which reflects the quality of the sampling.
- Its signal is detected at **670 nm** in **PCR2**.

4. CONTENT OF THE KIT AND STORAGE CONDITIONS

SARS-COV-2 R-GENE® 423735

Number of tests: 120 for **PCR1** and 30 for **PCR2**

There are enough reagents provided in this kit to perform a maximum of 8 PCR runs.

Before and after opening the kit, the reagents must be stored at -15°C/-31°C.

Once thawed, the use of a cooling block (+2°C/+8°C) is required for the handling of the reagents.

Reagents must be homogenized before use.

Reagents must be placed back to storage at -15°C/-31°C after use.

Designation	Name	Composition	Presentation	Storage Conditions
W0	Water (molecular grade)	-	2 x 1.8 mL	-
IC1 (green)	Internal control 1	Ready-to-use RNA internal control	2 x 1[illegible] mL	The **IC1** cannot undergo more than freeze/thaw cycles. It is stable for up to six hours at ≤ +30°C. Thereafter, internal control aliquoting is recommended.
R01 (red)	SARS-COV-2 amplification premix	Contains the dNTPs, $MgCl_2$, amplification buffer, primers and probes for the tested targets and internal control and Taq Polymerase. The **R01** amplification premix is used only for **PCR1**.	2 x 900 µL	**R01** cannot undergo more than 4 freeze/thaw cycles and must be kept away from light.
R02 (blue)	SARBECOVIRUS amplification premix	Contains the dNTPs, $MgCl_2$, amplification buffer, primers and probes for the tested targets and internal control, and Taq Polymerase. The **R02** amplification premix is used only for **PCR2**.	1 x 450 µL	**R02** cannot undergo more than 8 freeze/thaw cycles and must be kept away from light.
PC1 (purple)	SARS-COV-2 Positive control	2 Transcripts for SARS-CoV-2. The **PC1** is used only for **PCR1**.	1 x 110 µL	The **PC1** cannot undergo more than 8 freeze/thaw cycles.
PC2 (pink)	SARBECOVIRUS and Cc Positive control	1 Transcript for Sarbecovirus and 1 plasmid for the Cell Control. The **PC2** is used only for **PCR2**.	1 x 110 µL	The **PC2** cannot undergo more than 8 freeze/thaw cycles.

Designation	Name	Composition	Presentation	Storage Conditions
RT (orange)	Reverse Transcriptase Superscript® III (concentrated)	-	1 x 15 µL	**RT** is sensitive to temperature variations. It must be taken out just before use and returned to -15°C/-31°C as soon as possible.
1 package insert provided in the kit or downloadable from www.biomerieux.com/techlib.				

5. WARNINGS AND PRECAUTIONS

- For prescription use only.
- For *in vitro* diagnostic use only.
- For Emergency Use only.
  - This test has not been FDA cleared or approved;
  - This test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, 42 U.S.C. §263a, that meet requirements to perform high complexity tests;
  - This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
  - This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The kit must be handled by qualified personnel, in accordance with Good Laboratory Practice and handling instructions for molecular biology.
- Read all instructions before starting.

5.1. General Warnings and Precautions

- Avoid contact between reagents and skin. In case of contact, wash immediately with large amounts of water.
- Samples must be prepared under a biological safety hood.
- This kit contains products of animal origin. The certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Unused reagents may be considered as non-hazardous waste and disposed of accordingly. Dispose of used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
  It is the responsibility of each laboratory to handle waste and effluents produced, according to their nature and degree of hazardousness, and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.
- Do not use reagents after the expiration date indicated on the label.

5.2. Warnings and Precautions for Molecular Biology

- Amplification procedures require highly-skilled techniques to avoid risk of sample contamination:
  - Reagent preparation, sample preparation and amplification steps must be carried out in separate work areas. Movement in the laboratory must be in one direction only, from the reagent preparation area to the amplification area. Allocate a set of lab coats and pipettes to each area. Never introduce an amplified product in reagent and/or sample preparation areas.
  - Pipettes used to handle samples are reserved for this purpose only. The pipettes used to prepare and dispense reagents are also reserved for this purpose only.
  - In the case of manual use, tubes from different samples and amplification premixes must never be opened at the same time.
  - The samples used must be exclusively reserved for this analysis.
- Do not substitute reagents from kits with different batch numbers.
  - Exception: A sample extracted with the internal control **IC1** of this kit may be tested with another kit of the ARGENE range of products. If a sample extracted with the **IC1** from the SARS-COV-2 R-GENE® kit is used with another kit, the extracted **IC1W0** from that same extraction run must be used.
- Do not substitute reagents with those from other manufacturers.
- Do not use reagents of the amplification kit if these are thawed upon receipt.
- The reagents must be fully thawed to +18°C/+25°C prior to use and homogenized, except for **RT**, which must be taken out just before use, and returned to -15°C/-31°C as soon as possible.
- Once thawed, the use of a cooling block (+2°C/+8°C) is required for the handling of the reagents.

- Always perform preventive maintenance for workstations, for automated extraction, amplification platforms, and centrifuge systems, according to the manufacturer recommendations.

For more detailed information, see the product safety data sheet, which can be downloaded from www.biomerieux.com/techlib.

6. SAMPLE TRANSPORT, PREPARATION AND STORAGE

- Inappropriate sample collection, treatment, storage and/or transport could lead to erroneous results.
- Samples must be collected following the laboratory's instructions and transported in accordance with local regulations.
- Samples must be transported and processed by the laboratory in the shortest possible time (preferably within 24 hours).
- Samples must be transported in media compatible with molecular biology testing.

Upper Respiratory Specimens

Caution: The customer must follow the recommendations of the swab supplier. For example, the swab should not be used if the color of the medium has changed to light orange. This information is based on stability studies performed on COPAN swabs using Universal Transport Medium RT.

If the samples are not processed upon arrival, they may be stored for:

- 6 hours at +18°C/+25°C
- 72 hours at +2°C/+8°C
- 4 months at -15°C/-31°C or at ≤ -60°C with a maximum of 3 freezing/thawing cycles

Bronchoalveolar Lavage (BAL) specimens should be collected, transported and stored according to standard methods.

7. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

- Extraction systems validated with the kit; follow the manufacturer instructions (see the *PRINCIPLE OF THE TEST* section).
- Amplification platforms validated with the kit (see the *PRINCIPLE OF THE TEST* section).
- Centrifuge appropriate for the amplification platform.
- Tubes or plates appropriate for the amplification platform validated with the kit.
- Cooling block appropriate for the amplification platform validated with the kit.
- U.V. light.
- Workstation or plexiglass screen for sample and reagent mix distribution.
- Micropipettes and sterile filter tips appropriate for the volume to be pipetted.

8. SAMPLE EXTRACTION PROTOCOL

Caution: Before starting the extraction procedure, make sure thawed samples and reagent **IC1** have been homogenized.

8.1. Pretreatments Required Prior to Extraction

Upper Respiratory Samples

In case of extraction with the NUCLISENS® easyMAG® and EMAG® instruments, the samples (+ **IC1**) may require proteinase K pretreatment if the sample is considered to be too mucous. In this case, add 10 µL of Proteinase K (20 mg/mL) per 200 µL of sample and incubate for 15 minutes at +56°C.

8.2. Extraction Protocols Validated with the Kit

These extraction instruments must be regularly maintained by qualified personnel according to the manufacturer's recommendations.

Instruments	Kit	Test Sample	Type of Sample(1)	Protocol	Elution Volume
EMAG®	NUCLISENS® easyMAG® reagents	200 µL sample + 10 µL **IC1** (3)	Upper Respiratory Samples or BAL fluid	B31/B41(2)	50 µL
		400 µL sample + 10 µL **IC1**(3)			100 µL
NUCLISENS® easyMAG®	NUCLISENS® easyMAG® reagents	200 µL sample + 10 µL **IC1**	Upper Respiratory Samples or BAL fluid	Specific B with 50 µL silica	50 µL
		400 µL sample + 10 µL **IC1**			100 µL
MagNA Pure 96	DNA and Viral NA Large Volume Kit	250 µL sample + 10 µL **IC1** + 250 µL buffer (BLB for MagNA Pure 96)	Upper Respiratory Samples or BAL fluid	Pathogen Universal 500	50 µL

Instruments	Kit	Test Sample	Type of Sample(1)	Protocol	Elution Volume
QIAsymphony SP	QIAsymphony DSP Virus/Pathogen Mini Kit	200 µL sample + 10 µL **IC1**(3)	Upper Respiratory Samples or BAL fluid	Complex 200 OBL DSP	85 µL

(1) See the *PRE-TREATMENTS REQUIRED PRIOR TO EXTRACTION* section if applicable.

(2) For the extraction method code, refer to the EMAG® Extraction Method User Manual.

(3) **Note:** For automated systems, take into account the dead volume in the test run (sample and **IC1**).

Eluates must be stored as recommended by the manufacturer of the instrument. For BIOMÉRIEUX instruments, this information is provided in the instrument user manual.

9. REAL-TIME DETECTION PROTOCOL

Note: In order to simplify the instructions, the device dedicated to holding the amplification reaction mix is referred to as "**tube**".

- The products to be amplified correspond to the eluates obtained by the extraction methods validated with the kit.

9.1. Thermocycler Programming

- Regardless of which real-time PCR instrument is used, the amplification program is as follows:

Steps		Time	Temperature	Cycles	Fluorescence acquisition			
					LC480 (System II)	Validated Applied Biosystems	Rotor-Gene Q	CFX96
The temperature increases and decreases are set by default up to 20°C/sec. at 100%.								
Reverse Transcription		5 min.	50°C	1	-	-	-	-
Taq Polymerase Activation		15 min.	95°C	1	-	-	-	-
Amplification	Denaturation	10 sec.	95°C	45	-	-	-	-
	Annealing	40 sec.	60°C		**FAM (465-510)** **Cy5"/"Cy5.5" (618-660)** **VIC/HEX/ Yellow555 (533-580)**	**FAM** **"CY5"** **VIC**	**Green** **Red** **Yellow**	**FAM** **"Cy5"** **HEX**
					At end of annealing			
	Elongation	25 sec.	72°C		-	-	-	-

Note: On LightCycler 480, add a cooling step: 30 sec./40°C/1 cycle at the end of the PCR.

Note: On LightCycler 480, there are two optical systems: only "System II" is compatible with the use of the kit. "System II" includes automatic color compensation in its software.

Note: On Applied Biosystems, select "**NONE**" in "**PASSIVE REFERENCE**".

Note: On Rotor-Gene Q, to enable a correct reading of Cyanine 5 dye in red channel, when running several parameters together in the same PCR rotor:

- the positive control **PC2** must be placed in position 1.
- OR, in the Auto-Gain Optimisation Channel Settings tab, for the three channels (Red, Yellow and Green), select the Tube Position of the positive control **PC1** or **PC2**.

Programming guidelines are available upon request. Please contact your local BIOMÉRIEUX representative.

9.2. Amplification Preparation

Make sure that the cooling block was decontaminated (for example, by exposure to U.V. light for 30 minutes).

In the room reserved for amplification

- Before each use:
  - The reagents must be fully thawed at **+18°C/+25°C** before starting the assay.
  - Once thawed, the reagents must be kept on a cooling block (+2°C/+8°C).
  - Homogenize the **PC1** and/or **PC2** (using a vortex-type mixer for 5-10 seconds) and briefly centrifuge.
  - Homogenize the other reagents (using a vortex-type mixer for 2-5 seconds) and briefly centrifuge.

Caution: In case of manual pipetting, and to prevent contamination as much as possible, close the tubes when distribution is completed.

Caution: All reagents (amplification premix (R01 and R02), Positive Controls PC1 and PC2 and RT) must be returned to -15°C/-31°C immediately after use.

For each PCR (i.e., **PCR1** and/or **PCR2)**, prepare n tubes:

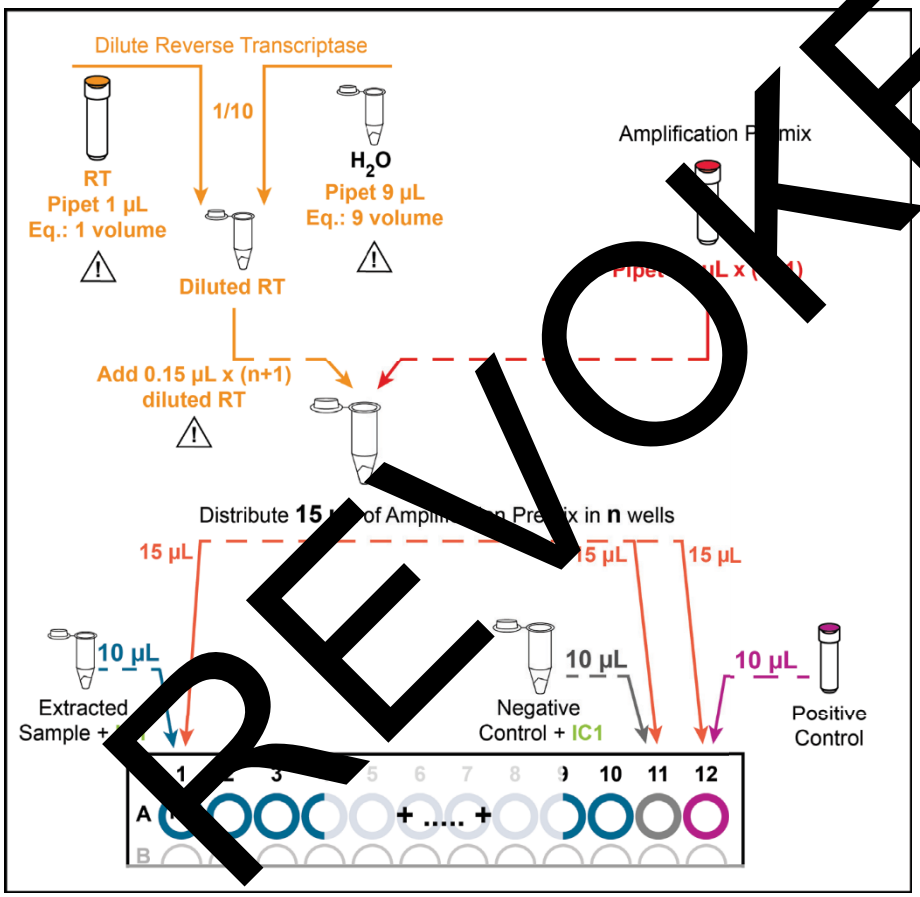
1	tube	per tested sample.
1	tube	for the positive amplification control (**PC**).
1	tube	for negative control (**IC1W0**).

Note: For the CFX96 amplification instrument, use the transparent plates with optical stoppers.

Note: For the LightCycler 480 System II amplification instrument, use the white LightCycler 480 Multiwell Plate 96.

Note: For the Rotor-Gene Q amplification instrument, use the "Strip Tubes and Caps, 0.1 mL".

Note: For the Applied Biosystems amplification instruments validated, use the "MicroAmp Optical Adhesive Film" and "MicroAmp Optical 96-well Reaction Plate" with block for 0.2 mL 96-well plates, or "MicroAmp Fast Optical 96-Well Reaction Plate, 0.1 mL" with block for 0.1 mL 96-well plates.



- Pipet (n + 1) x 15 µL of amplification premix specific to the targeted pathogen and transfer to a suitable tube (0.2 mL-0.5 mL).
- Add (n + 1) x 0.15 µL of **RT** diluted to 1:10 in water.

Note: When using an automated pipetting system, please refer to the instrument user manual to plan the amount of premix to prepare according to the number of samples to test.

Note: For the preparation of Superscript® III Reverse Transcriptase, suitable precision pipettes should be used. Do not pipette volumes less than 1 µL.

Caution: The 1/10 RT dilution must be prepared extemporaneously and immediately added to the amplification premix. Do not store diluted **RT**.

Example for 18 samples and 2 controls to be analyzed in one run: ***n*** *= 18 + 2 =* ***20***

Pipet 3 µL of ***RT*** *diluted in water + 300 µL of amplification premix specific to the targeted pathogen and transfer to a suitable tube.*

- Homogenize this reaction mixture briefly using a vortex-type mixer and briefly centrifuge.
- Draw and expel 15 µL in the reaction mixture prepared as described above.
- Distribute 15 µL of this mixture in each tube.
- When adding the sample, draw and expel to homogenize the reaction mixture (except for Rotor-Gene Q).

The following distribution order should be followed for manual procedures:

1. Add 10 µL of **IC1**+**W0** extract in the corresponding tube. This tube is the **IC1W0** control (see the *CONTROLS* section).
2. Add 10 µL of extracted sample in each corresponding tube.
3. Add 10 µL of Positive Control in the corresponding tube. This tube is the positive amplification control (see the *CONTROLS* section).
4. Centrifuge the tubes with the appropriate device, if applicable, and then transfer them to the thermocycler.
5. Launch the amplification program described in the *REAL-TIME DETECTION PROTOCOL* section.

10. RESULTS ANALYSIS

Programming guidelines are available upon request. Please contact your local bioMÉRIEUX representative.

530 nm = corresponds to the reading channel "FAM", "Green" or other, depending on the real-time PCR platforms. In order to simplify the instructions, only the term "**530 nm**" is used.

560 nm = corresponds to the reading channel "VIC", "Hex", "YELLOW" or other, depending on the real-time PCR platforms. In order to simplify the instructions, only the term "**560 nm**" is used.

670 nm = corresponds to the reading channel "CY5", "Red" or other, depending on the real-time PCR platforms. In order to simplify the instructions, only the term "**670 nm**" is used.

Ct = Cycle Threshold for most real-time PCR platforms (**C**rossing **P**oint) for the LightCycler range of instruments.

10.1. Data Analysis with LightCycler 480 (System II)

- Switch on the LC480 **FAM - HEX** automatic compensation.
- For the analysis in "**CY5**", select **OFF** in **COLOR COMPENSATION**.
- The targets are analyzed in **ABSOLUTE QUANTIFICATION** mode at **530 nm (FAM)** and at **670 nm (Cyanine 5)**.
- The extraction + inhibition controls are analyzed in **ABSOLUTE QUANTIFICATION** mode at **560 nm (HEX)**.
- For each positive sample, a **CROSSING POINT** (CP) is calculated at **530 nm** and **670 nm**.
- The extraction + inhibition controls are analyzed by comparing the calculated CP value for each extraction + inhibition control (**IC1sample**) with the CP value obtained with the reference extraction + inhibition control (**IC1W0**) at **560 nm (HEX)**.

Use the **FIT POINTS** method to determine the status (positive/negative) of the samples and controls.

Analysis using Fit Points:

Analysis using **FIT POINTS** consists of three steps: **STEP 1: CYCLE RANGE**; **STEP 2: NOISE BAND**; **STEP 3: ANALYSIS**.

In **STEP 1**, establish the placement of the threshold (above background noise and in the exponential phase of each amplification curve, generally corresponding to 5–10% of the sample's final fluorescence). In **STEP 2**, report the estimated value so that the horizontal line eliminates the baseline noise and crosses all the curves at the beginning of their exponential phase. Then, in **STEP 3**, report the value in order to place the threshold line.

Move the threshold line for each target.

10.2. Data Analysis on Validated Applied Biosystems

- Check that **NONE** has been selected in the **PASSIVE REFERENCE** field, since the amplification premix does not contain any passive reference fluorochrome.
- The targets are analyzed after having selected the **FAM** and **Cyanine 5** detector/reporter in the **DETECTOR/ REPORTER** field.
- The extraction + inhibition (**IC1sample** and **IC1W0**) controls are analyzed after having selected the VIC detector/ reporter in the **DETECTOR/REPORTER** field.

Note: Detectors/targets can be created for each tested parameter (e.g.: **CMV**, **IC2 CMV**, etc.). To do so, create a new detector/reporter by selecting **FAM** or **Cyanine 5** as **REPORTER** and **NONE** or **NFQ-MGB** as **QUENCHER**.

- The extraction + inhibition controls are analyzed by comparing the calculated Ct value for each extraction + inhibition control (**IC1sample**) with the Ct obtained with the reference extraction + inhibition control (**IC1W0**) at **560 nm**.
- For each positive sample, a Ct is calculated at **530 nm (FAM)** or **670 nm (Cyanine 5)**. Negative samples or controls defined as **UNDETERMINED** are displayed in the **Ct** column.
- In linear visualization mode (uncheck automatic method), manually move the threshold line so that it is:
  - above the baseline noise
  - in the exponential phase of each amplification curve, generally corresponding to 5–10% of the sample's final fluorescence.

Note:
- Move the threshold line for each target.
- Check the **PC** as defined in the *VALIDATION AND INTERPRETATION OF RESULTS* section.

10.3. Data Analysis on Rotor-Gene Q

Note: When running several parameters together in the same PCR rotor, the positive control must be placed in position 1 (to enable correct reading of Cyanine 5 dye in the red channel).

- The targets are analyzed in **CYCLING A GREEN** mode at **530 nm** or **CYCLING A RED** at **670 nm**.
- The extraction + inhibition controls are analyzed in **CYCLING A YELLOW** mode at **560 nm**.
- For each positive sample, a **CYCLE THRESHOLD** (Ct) is calculated at **530 nm (green)** or **670 nm (red)**.
- The extraction + inhibition controls are analyzed by comparing the calculated Ct value for each extraction + inhibition control (**IC1sample**) with the Ct obtained with the reference extraction + inhibition control (**IC1W0**) at **560 nm (Yellow)**.

Note: Analysis pages **(PAGE)** can be created for each tested parameter. In this case, select the corresponding page.

- Select the **DYNAMIC TUBES** option.
- If the baseline fluorescence level is not constant, select the **SLOPE CORRECT** option.
- If the first cycle(s) is(are) not representative (fluorescence signal drop, random fluorescence variations, etc.), select the **IGNORE FIRST** option in order to delete it (them).

Note: The **SLOPE CORRECT** and **IGNORE FIRST** options are not necessarily mandatory and cumulative.

- In **LINEAR SCALE** mode, manually move the threshold line so that it is:
  - above the baseline noise
  - in the exponential phase of each amplification curve, generally corresponding to 5–10% of the sample's final fluorescence.

Note:
- Move the threshold line for each target.
- Check the **PC** as defined in the *VALIDATION AND INTERPRETATION OF RESULTS* section.

10.4. Data Analysis on CFX96

- The targets are analyzed under the **QUANTITATION** tab by leaving only the **FAM** and "**Cy5**" buttons checked.
- The extraction + inhibition controls are analyzed under the **QUANTITATION** tab by leaving only the **HEX** button checked.
- The extraction + inhibition controls are analyzed by comparing the calculated Ct value for each extraction + inhibition control (**IC1sample**) with the Ct obtained with the reference extraction + inhibition control (**IC1W0**) at **560 nm**. Optional: Select a premix in the **FLUOROPHORE** drop-down menu if this has been previously created.
- In **SINGLE THRESHOLD** mode, manually move the threshold line so that it is:
  - above the baseline noise
  - in the exponential phase of each amplification curve, generally corresponding to 5–10% of the sample's final fluorescence.
- For each positive sample, a Ct is calculated. Negative samples or controls are indicated by **N/A** in the **Ct** column.

Note:
- Move the threshold line for each target.
- Check the **PC** as defined in the *VALIDATION AND INTERPRETATION OF RESULTS* section.

11. VALIDATION AND INTERPRETATION OF THE RESULTS

	530 nm	560 nm	670 nm
PCR1	Ct for N gene	Ct for **IC1**	Ct for RdRp gene
PCR2	Ct for E gene	Ct for **IC1**	Ct for Cell Control

11.1. PCR Run Validation

Caution: The run is only valid if all the following conditions are met. If this is not the case, refer to the *TROUBLESHOOTING* section.

Ct = Cycle Threshold for most real-time PCR platforms or CP (Crossing Point) for the LightCycler range of instruments.

	Run validation conditions for PCR1		
	530 nm	**560 nm**	**670 nm**
IC1W0	NEG (no Ct)	Ct ≤ 36	NEG (no Ct)
PC1(1)	Ct < 34	-	Ct < 34

	Run validation conditions for PCR2		
	530 nm	**560 nm**	**670 nm**
IC1W0	NEG (no Ct)	Ct ≤ 35	Ct > 35 or NEG (no Ct)
PC2(1)	Ct < 34	-	Ct < 34

(1) **IC1** is not added to the PCR for **PC1** or **PC2**: no Ct value for **IC1** should be obtained.

⇒ If all of these conditions are met, the run is valid, and the results can be read.

11.2. Interpretation of the Results

- Each sample must be analyzed individually.
- For **PCR1**, a Ct value is displayed at **530 nm** or **670 nm** for all positive samples.
- For **PCR2**, a Ct value is displayed at **530 nm** for all positive samples.
- The absence of inhibition must be checked as well as the proper execution of the extraction step at **560 nm**, according to the elements described below.

Results interpretation for PCR1 (SARS-CoV-2)

	Ct target or ΔCt [IC1sample- IC1W0]				
N Gene (**530 nm**)	+(1)	+(1)	-	-	
IC1sample - **IC1W0** (**560 nm**)	Any difference in Ct value is acceptable			**≤ 3 Ct**	**> 3 Ct**
RdRp Gene (**670 nm**)	+(1)	-	+(1)	-	
	INTERPRETATION				
	POSITIVE for SARS-CoV-2 RNA			SARS-CoV-2 RNA NOT detected(2)	Invalid result (inhibition/poor extraction)(3)

(1) Any Ct value is considered as positive result.

(2) SARS-CoV-2 RNA not present or below LoD. In case of unexpected negative result, sample eluate can be tested with **PCR2** to check sample quality (presence of cells).

(3) Invalid due to extraction issue or inhibition effect. Recommendation to test new sample.

Results interpretation for PCR2 (Sarbecovirus)

	Ct target or ΔCt [IC1sample - IC1W0]			
E Gene (**530 nm**)	**+**(1)	**-**		**-**
IC1sample - **IC1W0** (**560 nm**)	**Any difference in Ct value is acceptable**	**≤ 3 Ct**		**> 3 Ct**
Cell Control (**670 nm**)	**+ or -**	**< 35 Ct**	**≥ 35 Ct or -**	**+ or -**
	INTERPRETATION			
	Presumptive POSITIVE for SARS-COV-2 RNA(2)	SARS-CoV-2 RNA NOT detected(3)	Cells NOT detected(4)	Invalid result (inhibition/poor extraction)(5)

(1) Any Ct value is considered as positive result.

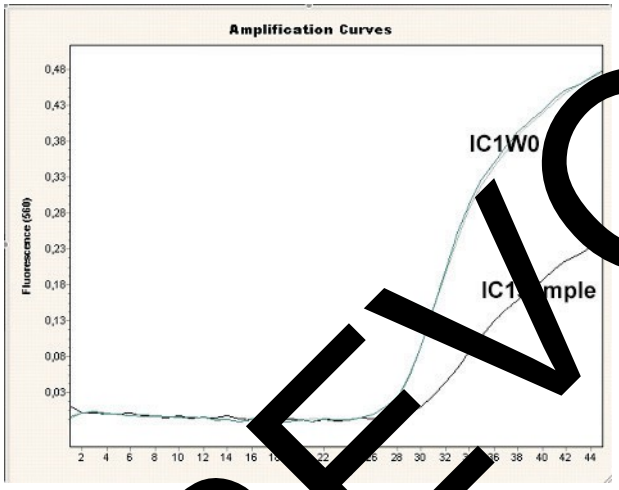
(2) E gene allows for detection of Sarbecovirus. Because SARS-CoV is currently not circulating in the US, a positive result for the E gene is regarded as presumptive positive for the presence of SARS-CoV-2 RNA.

(3) SARS-CoV-2 RNA not present or below LoD.

(4) Sample collection was not made adequately, recommendation to test new sample.

(5) Invalid due to extraction issue or inhibition effect. Recommendation to test new sample.

If the slope of the curve generates a drop in final fluorescence ≥ 50% compared with final fluorescence **IC1W0** (see figure below), this represents an inhibition. Test the sample again.



12. LIMITATIONS

The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

The performance of the SARS-COV-2 R-GENE® assay was established using contrived nasopharyngeal swab samples. Oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid are also considered acceptable specimen types for use with the SARS-COV-2 R-GENE® assay. Please refer to the FDA FAQs on Diagnostic Testing for SARS-CoV-2 for additional information regarding acceptable specimen types for detection of SARS-CoV-2.

Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

False-negative results may arise from:

- Improper sample collection
- Degradation of the viral RNA during shipping/storage
- Using unauthorized extraction or assay reagents
- The presence of RT-PCR inhibitors
- Mutation in the SARS-CoV-2 virus
- Failure to follow instructions for use

False-positive results may arise from:

- Cross contamination during specimen handling or preparation
- Cross contamination between patient samples
- Specimen mix-up
- RNA contamination during product handling

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.

A positive result for either the N or RdRp targets indicates the detection of nucleic acid from SARS-CoV-2. A positive result from the E-gene target should be considered presumptive for the detection of SARS-CoV-2 virus.

Nucleic acid may persist even after the virus is no longer viable.

Laboratories are required to report all positive results to the appropriate public health authorities.

13. CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The SARS-COV-2 R-GENE® test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:
https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

However, to assist clinical laboratories running the SARS-COV-2 R-GENE® test, the relevant Conditions of Authorization are listed below:

1. Authorized laboratories[1] using the SARS-COV-2 R-GENE® test will include with result reports of the SARS-COV-2 R-GENE® test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
2. Authorized laboratories using the SARS-COV-2 R-GENE® test will use the SARS-COV-2 R-GENE® test as outlined in the SARS-COV-2 R-GENE® Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the SARS-COV-2 R-GENE® test are not permitted.
3. Authorized laboratories that receive the SARS-COV-2 R-GENE® test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
4. Authorized laboratories using the SARS-COV-2 R-GENE® test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and bioMérieux local technical support center (800-682-2[illegible] or CustomerService-ImmunoMolecular@biomerieux.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
6. All laboratory personnel using the test must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
7. bioMérieux SA, authorized distributors, and authorized laboratories using the SARS-COV-2 R-GENE® test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

14. PERFORMANCE

Caution: The performance described has been validated and is guaranteed using the extraction and amplification systems combinations described in this section.

14.1. Analytical Sensitivity

The analytical sensitivity of the SARS-COV-2 R-GENE® kit was determined using serial dilutions of inactivated SARS-CoV-2 viral culture (BetaCoV/France/IDF0571/2020, GISAID EPI ISL 411219, provided by VIRPATH laboratory, Lyon, France), diluted in lysed negative clinical nasopharyngeal swabs. Determination was done on 4 concentrations with 5 replicates per concentration. The lowest concentration with 100% hit rate was considered the estimated limit of detection (LoD) and confirmed by testing 20 replicates, along with a lower concentration to demonstrate a hit rate below 95%. All experiments were done with both **PCR1** for the N and RdRp genes (SARS-CoV-2 detection target) and **PCR2** for the E gene (Sarbecovirus detection target). Extractions were performed on NUCLISENS® easyMAG® and EMAG®, and the amplifications were performed on Applied Biosystems 7500 Fast Dx.

As a result, the **claimed limit of detection for SARS-COV-2 R-GENE® is 0.43 TCID 50/mL, equivalent to 380 genomic copies/mL (based on quantification by digital RT-PCR)**.

Based on Ct values and hit rates at lower concentration, sensitivity was shown to be slightly better for the N gene compared to the E gene and to the RdRp gene.

Evaluation of Alternative Nucleic Acid Extraction and Amplification Systems

To evaluate the compatibility of the SARS-COV-2 R-GENE® assay with alternative nucleic acid extraction technologies and PCR instrument systems, testing was performed using contrived nasopharyngeal swab specimens that were spiked with different concentrations of inactivated SARS-CoV-2. For each combination of extraction and amplification platforms, the conditions tested included at least 6 replicates at the claimed LoD target concentration of 0.43 TCID 50/mL. In all cases, all 6 replicates at the LoD concentration produced the expected results for the N and RdRp gene targets in **PCR1** and the E gene target in **PCR2**. Samples that were tested at higher concentrations of SARS-CoV-2 also produced the expected results. Testing included the combinations of extraction systems and PCR instruments listed in the table below and showed that the analytical sensitivity of the assay was similar, irrespective of the combination of extraction and amplification platforms used. These results support use of the specified nucleic acid extraction and PCR amplification systems interchangeably to perform the SARS-COV-2 R-GENE® assay.

		Extraction			
		EMAG®	QIASymphony SP	MagNA Pure Compact	MagNA Pure 96
PCR	ABI 7500 Fast Dx	X	X	X	X
	LightCycler 480 (System II)	X			
	Rotor-Gene Q	X			
	Bio-Rad CFX96	X			
	QuantStudio 5 Dx	X			

X: combination tested

14.2. Precision

The precision study was conducted using the SARS-COV-2 R-GENE® kit via a repeatability study (within-run variation) and a reproducibility study (within-laboratory variation) on a panel of samples (2 different concentrations of inactivated SARS-CoV-2 diluted in a pool of clinical nasopharyngeal swabs and 1 negative sample). Each panel of samples was tested with 2 lots of reagents over 5 days, with 3 days per lot, 1 run per day, and 3 replicates per run for a total of 18 replicates per concentration. The extraction was performed using EMAG® and amplification on Applied Biosystems 7500 Fast Dx.

All negative samples returned negative and all 18 replicates returned positive per each concentration tested.

The table below shows the mean Ct values obtained for the 18 tests repeated for each positive sample. The standard deviation and coefficient of variation for repeatability and reproducibility were determined. The results of Ct values for Cell Control obtained for all samples including negative samples are also indicated.

Sample	Target	Mean Ct Value	Variability Source	Standard Deviation	Coefficient of Variation (%)
LoD	N gene	36.9	Reproducibility (1)	0.73	2.0
			Repeatability (2)	0.60	1.6
5xLoD		32.8	Reproducibility	0.49	1.5
			Repeatability	0.16	0.5

Sample	Target	Mean Ct Value	Variability Source	Standard Deviation	Coefficient of Variation (%)
LoD	RdRp gene	36.4	Reproducibility	0.99	2.7
			Repeatability	0.76	2.1
5xLoD		31.8	Reproducibility	0.32	1.0
			Repeatability	0.21	0.7
LoD	E gene	38.4	Reproducibility	0.93	2.4
			Repeatability	0.91	2.4
5xLoD		33.8	Reproducibility	0.43	1.3
			Repeatability	0.22	0.7
All (Negative, LoD and 5 x LoD)	Cell Control	29.4	Reproducibility	0.40	1.4
			Repeatability	0.3	1.2

⇒ These values demonstrate the acceptable repeatability and reproducibility for the kit.

(1) Within laboratory reproducibility

(2) With run repeatability

14.3. Analytical specificity

14.3.1. Inclusivity

The inclusivity of the primers and probes for the detection of SARS-CoV-2 was determined through *in silico* analysis of available sequences in NCBI & GISAID databanks.

For **PCR1** (N gene detection), 100% homology was shown for 99.7% of sequences from NCBI (n=316/317). The last sequence had 1 mismatch but was predicted to be detectable. 100% homology was shown for 99% of sequences from GISAID (n=10537/10648). 101 sequences had less than 2 mismatches (or indeterminate bases) leading to predicted detectability. For 1 sequence, no detection was predicted, however this sequence was shown to be 100% homologous with the RdRp gene primers and probe of **PCR1**. The remaining 9 sequences belong to SARS-CoV-2-like sequences of animal origin.

For **PCR1** (RdRp gene detection), 100% homology was shown for all sequences from NCBI (n=381). 100% homology was shown for 99.6% of sequences from GISAID (n=10787/10827). 34 sequences had less than 2 mismatches (or indeterminate base) leading to predicted detectability. For 1 sequence, no detection was predicted; this sequence was shown to be 100% homologous with the N gene primers and probe of **PCR1**. The remaining 7 sequences belong to SARS-CoV-2-like sequences of animal origin.

For **PCR2** (E gene detection), 100% homology was shown for 98.7% of sequences from NCBI (n=367/372), the 5 other sequences present less than 2 mismatches per oligonucleotide, leading to predicted detectability. 100% homology was shown for 99.1% of sequences from GISAID (n=10685/10779). For all but 3 sequences with a non-perfect match, no impact on detection is expected. In all cases, whenever a sequence had a non-perfect match, it was shown to be detectable with at least one of the 2 targets of **PCR1**.

14.3.2. Exclusivity and Biological Interference

The specificity of the primers and probes for SARS-COV-2 R-GENE® was demonstrated through *in silico* analysis and experimentally on pathogens that are reasonably likely to be encountered in nasopharyngeal swabs. The extraction was performed with the NUCLISENS® easyMAG® and the amplification on an Applied Biosystems 7500 Fast Dx.

The pathogens listed below were added at high concentrations:

- in negative nasopharyngeal swabs (exclusivity study).
- in nasopharyngeal swabs in which the SARS-CoV-2 virus was added at 3xLoD, i.e., 1.29 TCID 50/mL (biological interference study).

PCR1: No cross reaction and no interferences were reported for the following pathogens:

- **Viruses**: Human coronavirus: 229E, NL63, OC43, HKU1, SARS and MERS-coronavirus, Adenovirus, Human Metapneumovirus (hMPV), Parainfluenza virus 1-4, Influenza A, Influenza B, Enterovirus, Respiratory syncytial virus (RSV), Parechovirus and Rhinovirus.
- **Bacteria**: *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Bordetella pertussis*, *Mycoplasma pneumoniae*

PCR2: No cross reaction and no interferences were reported for the following pathogens:

- **Viruses**: Human coronavirus: 229E, NL63, OC43, HKU1, MERS-coronavirus, Adenovirus, Human Metapneumovirus (hMPV), Parainfluenza virus 1-4, Influenza A, Influenza B, Enterovirus, Respiratory syncytial virus (RSV), Parechovirus and Rhinovirus.
- **Bacteria**: *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Bordetella pertussis*, *Mycoplasma pneumoniae*

14.4. Clinical Performance Study

The clinical evaluation of the SARS-COV-2 R-GENE® kit was conducted with clinical nasopharyngeal swabs in universal transport medium. Swabs were contrived with inactivated SARS-CoV-2 and tested with the SARS-COV-2 R-GENE® assay to generate the Positive Percent Agreement (PPA) and, Negative Percent Agreement (NPA) as estimate of diagnostic accuracy.

50 nasopharyngeal swabs were used for diagnostic accuracy assessment of the SARS-CoV-2 target and 76 nasopharyngeal swabs were used for negative percent agreement evaluation.

These samples were extracted with the EMAG® instrument, following the B31 extraction method protocol, then amplified on the Applied Biosystems 7500 Fast Dx with both **PCR1** (N and RdRp genes) and **PCR2** (E gene) of the SARS-COV-2 R-GENE® kit.

The results are presented in the following table:

		SARS-CoV-2 Contrived Nasopharyngeal Swabs			
		[1-2]xLoD	> 2xLoD	Negative	**Total**
SARS-COV-2 R-GENE® (**PCR1** and **PCR2**)	[1-2]xLoD	20	0	0	20
	> 2xLoD	0	30	0	30
	Negative	0	0	76	76
Total		20	30	76	126

All 50 SARS-CoV-2 contrived positive nasopharyngeal swabs were found positive with both **PCR1** (N and RdRp genes) and **PCR2** (E gene). All 76 negative samples were found negative with both **PCR1** (N and RdRp genes) and **PCR2** (E gene).

As a conclusion, for each target (N & RdRp gene for **PCR1**, and E gene for **PCR2**), there was 100% positive agreement and 100% negative agreement.

An additional clinical evaluation was conducted with 60 frozen nasopharyngeal swabs in universal transport medium amongst which 30 were positive clinical samples collected from patients suspected of SARS-CoV-2 infection during the COVID-19 disease and 30 were collected from patients suspected of respiratory infection prior to the COVID-19 disease (and therefore considered to be negative for SARS-CoV-2 RNA). Samples were tested in a blinded fashion with both SARS-COV-2 R-GENE® kit and with BIOFIRE® COVID-19 test.

For testing with SARS-COV-2 R-GENE®, samples were extracted on the EMAG® instrument and amplified on the CFX96. For testing with BIOFIRE® COVID-19 tests, pouches were run on FILMARRAY® 2.0 instrument.

All 30 samples that were SARS-CoV-2 positive with the BIOFIRE assay returned positive results with the SARS-COV-2 R-GENE® with both PCR1 and PCR2. All 30 samples that were SARS-CoV-2 negative with the BIOFIRE assay also returned negative results with the SARS-COV-2 R-GENE® for both PCR1 and PCR2.

		BIOFIRE® COVID-19		
		Positive	Negative	Total
SARS-COV-2 R-GENE® PCR1 and PCR2	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Agreement		100% (30/30); 88.7-100% [1]		
Negative Agreement		100% (30/30); 88.7-100%		

[1] Two-sided 95% score confidence interval

As a result, there was 100% positive and negative agreement between SARS-COV-2 R-GENE® and BIOFIRE® COVID-19 assays.

14.5 FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded samples testing was used to establish the specificity and to confirm the LoD. The study was performed on EMAG® and Bio-Rad CFX96 system. The results are summarized in the following table:

Reference material provided by FDA	Specimen type	Product LoD	Cross-reactivity
SARS-CoV-2	NP swab	18 000 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not Applicable

ND: Not Detected

15. TROUBLESHOOTING

15.1. Pathogen Not Detected in Positive Samples

Possible Causes	Solutions
Alteration of the amplification premix.	• Follow instructions in the *CONTENT OF THE KIT AND STORAGE CONDITIONS* section. The premixes must not be thawed more than the number of times indicated in this section. • Check that the amplification premixes and positive control have been returned to -15°C/-31°C immediately after each use. • Check that the amplification premixes and positive control have been thawed at +18°C/+25°C. • Use a cooling block (+2°C/+8°C) when preparing and dispensing the premixes. • **RT** is sensitive to temperature variations. It must be taken out just before use and returned to -15°C/-31°C as soon as possible.
Problem with sample collection, transport and storage conditions in the laboratory.	• Follow instructions in the *SAMPLE PREPARATION AND TRANSPORT* section, which defines the optimal conditions (temperature, time) for transport and storage. • Check the delay between the sample collection and its analysis.
Problem with storage conditions and expiration date of ARGENE® kits.	• Follow instructions in the *CONTENT OF THE KIT AND STORAGE CONDITIONS* section. ARGENE® kits must be stored at -15°C/-31°C and kept away from light.

Possible Causes	Solutions
Extraction problem.	• Check that the samples were thoroughly mixed before the extraction was performed. • Check the materials and protocols used to extract samples. Kit performance is only validated for extractions described in the *SAMPLE EXTRACTION PROTOCOL* section. • Always perform preventive maintenance of devices for automated extraction according to the manufacturer recommendations. • The **IC1** used with **W0** and the sample must come from the same batch.
Reagent and sample distribution error.	• Check the calibration of pipettes. • Check that the correct volumes are used. • For kits including Reverse Transcriptase, check that it was well diluted before being added to the premix (see the *REAL-TIME DETECTION PROTOCOL* section or *REAL-TIME DETECTION/REAL-TIME QUANTIFICATION PROTOCOL* section). • Ensure that the reagents and samples are thoroughly homogenized before dispensing in tubes.
Programming error.	• Check all programming parameters entered (detection channel, mode, number of cycles, temperature, time, reaction volume). Programming sheets are available on request. Please contact your local BIOMÉRIEUX representative. • Check all the steps regarding the entry of samples. • On Rotor-Gene Q, it is impossible to detect reading of Cyanine 5 dye in red channel when running several parameters together in the same PCR rotor. → the positive control **PC1** or **PC2** must be placed in position 1. → OR, in the Auto Gain Optimisation Channel Settings tab, for the three channels (Red, Yellow and Green), select the Tube Position of the positive control **PC1** or **PC2**.
Amplification problem.	• Check the thermal performance of the instrument as recommended by the manufacturer. • Always perform preventive maintenance of the real-time PCR instrument according to the manufacturer recommendations. • Check that the tubes are closed and the locking ring of the Rotor-Gene Q carousel is properly locked. • If using plates, ensure the placement and adhesion of the sealing tape. • Check that the disposables used are those recommended in the *REAL-TIME DETECTION/REAL-TIME QUANTIFICATION PROTOCOL* section.
Results analysis error.	• Check the threshold line adjustment. • If amplifying with Rotor-Gene Q, use raw data in case of doubt ("creeping" curves). Programming sheets are available on request. Please contact your local BIOMÉRIEUX representative.

Possible Causes	Solutions
Results interpretation error.	• Check that ALL the validation criteria have been met (see the *VALIDATION AND INTERPRETATION OF RESULTS* section). • With Applied Biosystems: ◦ Check that the absence of reference fluorochrome has been taken into account (**NONE** selected in the **PASSIVE REFERENCE** field). **Note:** If simultaneously using kits containing and not containing ROX, perform the two types of analysis (with and without passive reference) appropriate for each situation/kit. • Check that the results obtained have been corrected via a color compensation file in the LightCycler 480 instrument. • Compare the result of the extraction + inhibition control (**ICsample**) of the suspected sample with the result of the negative extraction + inhibition control (**ICW0**) (see the *VALIDATION AND INTERPRETATION OF RESULTS* section). Dilute the sample if necessary.

15.2. Pathogen Detected in Samples Characterized Negative

Possible Causes	Solutions
Contamination during experiment.	• Follow all recommendations in the *WARNINGS AND PRECAUTIONS* section. • Decontaminate the cooling blocks using, for example, U.V. light. • Follow the instructions provided by the manufacturer for the maintenance of extraction and amplification instruments. • ARGENE reagents and disposables must be handled by qualified personnel.
Reagent and sample distribution error.	• Check the calibration of pipettes. • Check that the correct volumes are distributed. • Ensure that the reagents and samples are thoroughly homogenized before dispensing in tubes.
Programming error.	• Check all programming parameters entered (detection channel, mode, number of cycles, temperature, time, reaction volume). Programming sheets are available on request. Please contact your local BIOMÉRIEUX representative. • Check all the steps regarding the entry of samples.
Results analysis error.	• Check the threshold line adjustment. • If amplifying with Rotor-Gene Q, use raw data in case of doubt ("creeping" curves). Programming sheets are available on request. Please contact your local BIOMÉRIEUX representative.

Possible Causes	Solutions
Results interpretation error.	• Check that ALL the validation criteria have been met (see the *VALIDATION AND INTERPRETATION OF RESULTS* section). • With Applied Biosystems: ◦ Check that the absence of reference fluorochrome has been taken into account (**NONE** selected in the **PASSIVE REFERENCE** field). **Note:** If simultaneously using kits containing and not containing ROX, perform the two types of analysis (with and without passive reference) appropriate for each situation/kit. • Check that the results obtained have been corrected via a color compensation file (**FAM** and **HEX**) in the LightCycler 480 instrument. • Compare the result of the extraction + inhibition control (**ICsample**) of the suspected sample with the result of the reference extraction + inhibition control (**ICW0**) (see the *VALIDATION AND INTERPRETATION OF RESULTS* section). Dilute the sample if necessary.
Cell Control detection in **IC1W0**.	• Detection of Cell Control may occur occasionally leading to a weak signal (Ct value > 35) due to the presence of gDNA in the culture media used to produce the Internal Control. This does not affect the interpretation of the results.

15.3. The Samples Are All Inhibited

Possible Causes	Solutions
Extraction problem.	• Check that the samples were thoroughly mixed before the extraction was performed. • Check the materials and protocols used to extract samples. • Kit performance is only validated for extractions described in the *SAMPLE EXTRACTION PROTOCOL* section. Always perform preventive maintenance of devices for automated extraction according to the manufacturer recommendations.
The **ICW0** does not result from the same extraction run.	• Check that the **IC** extracted with **ICW0** is from the same batch as the **IC** extracted with the tested sample. Each extraction run should have its own **ICW0**.

16. INDEX OF SYMBOLS

Symbol	Meaning
	Catalogue number
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Date of manufacture
	Contains sufficient for <n> tests
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Keep away from light
	For US Only: Caution: US Fede[illegible] restricts this device to sale by [illegible] on the order of a licensed practition[illegible]

17. LIMITED WARRANTY

bioMérieux warrants the performance of the pr[illegible]duct fo[illegible]s stated [illegible]tended use provided that all procedures for usage, storage and handling, shelf life (when applicabl[illegible] and pre[illegible]ons are strictly followed as detailed in the instructions for use (IFU).

Except as expressly set forth ab[illegible]ve, bio[illegible]ux he[illegible]by disclaims all warranties, including any implied warranties of merchantability and fitness f[illegible] particular purp[illegible] use, and disclaims all liability, whether direct, indirect or consequential, for any use [illegible] the reagent, software, instrument and disposables (the "System") other than as set forth in the IFU.

18. REVISION HISTORY

Change type cat[illegible]s

N/A	Not ap[illegible]le (First publication)
Correctio[illegible]	Correction of documentation anomalies
Technical ch[illegible]	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2020-05	055837-01	N/A	Not applicable (First publication)
2020-09	055837-02	Technical Change	Clinical Performance Study, FDA SARS-CoV-2 Reference Panel Testing

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