EURORealTime SARS-CoV-2

Instruction for Use

For prescription use only. For use under emergency use authorization only.

ORDER NO.	PARAMETER	FORMAT
MP 2606-0125 MP 2606-0225 MP 2606-0425 MP 2606-0100 MP 2606-0200	SARS-CoV-2	25 reactions 50 reactions 100 reactions 100 reactions 200 reactions

CE IVD

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Intended use

EURORealTime SARS-CoV-2 is a reverse transcriptase real-time polymerase chain reaction (RT real-time PCR) intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swabs) and bronchioalveolar lavage (BAL), from individuals suspected of COVID-19 by their health care provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and BAL during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of the disease.

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 EURORealTime SARS-CoV-2 is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The EURORealTime SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and explanation

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV) belongs to the family of coronaviruses and, like SARS-CoV, is classified in the genus Betacoronavirus [1]. The new coronavirus originated in China in the city of Wuhan, Hubei province. It caused an infection wave, which has spread rapidly within the country and worldwide [2, 3]. Just a few days after the first report about patients with pneumonia of unclear origin, the causative pathogen was identified as SARS-CoV-2 [2-5].

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected persons [2-4, 6]. Health care personnel and family members are especially at risk of infection [6, 7]. The zoonotic reservoir of the virus appears to be bats [2, 4, 6].

The incubation time of SARS-CoV-2 is three to seven, maximally 14 days [2]. The symptoms of SARS-CoV-2 infection are fever, coughing, breathing difficulties and fatigue [2-4, 6]. Some patients, especially elderly or chronically ill patients, develop acute respiratory distress syndrome (ARDS) [2, 3, 5, 6]. The fatality rate is between 0.6% and 7.2%, depending on the country [5]. In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO.

The detection of viral RNA is the most sensitive method to aid in the diagnosis of SARS-CoV-2 infections.

Test principle

The EURORealTime SARS-CoV-2 test detects SARS-CoV-2 RNA extracted from upper respiratory and BAL specimens. An RNA sequence that is unrelated to the SARS-CoV-2 sequence is added to each specimen at the beginning of sample preparation and is simultaneously amplified with the SARS-CoV-2 specific target sequences to serve as an internal control (IC). This control monitors correct sample processing and amplification for each sample and the negative control. The test combines reverse transcription (RT) to convert viral RNA into complementary DNA (cDNA) with PCR amplification and fluorescence-based real-time detection of two defined sections within the ORF1ab- and N-genes of the SARS-CoV-2 genome. Reverse transcription, amplification and detection of SARS-CoV-2 cDNA are carried out by means of SARS-CoV-2-specific primers and probes. The test contains an internal amplification control which serves as an inhibition control and can additionally be used as an extraction control. The test kit includes a SARS-CoV-2-positive control that is used as an external control in every test run.

Contents of the test kit

(MP 2606-####)

Component	0125	0225	0425	0100	0200	Symbol
1. PCR Mix A SARS-CoV-2 (green cap), ready for use	1 x 150 µl	2 x 150 µl	4 x 150 µl	1 x 600 µl	1 x 1.2 ml	PCR MIX A
2. PCR Mix B SARS-CoV-2 (yellow cap), ready for use	1 x 150 µl	2 x 150 µl	4 x 150 µl	1 x 600 µl	1 x 1.2 ml	PCR MIX B
3. Positive control SARS-CoV-2 (purple cap), ready for use	1 x 400 µl	1 x 400 µl	1 x 400 µl	1 x 400 µl	1 x 400 µl	POS CONTROL
4. RNA internal control (white cap), ready for use	1 x 1.2 ml	1 x 1.2 ml	1 x 1.2 ml	1 x 1.2 ml	1 x 1.8 ml	INT CONTROL
5. Instruction for use	1 piece	1 piece	1 piece	1 piece	1 piece	-

Test kit materials for re-ordering

The following materials can be re-ordered. They should always be used according to the test instruction of the test kit.

Material	Order number	Format
Positive control SARS-CoV-2	MK 2606-0108	1 x 400 µl
RNA internal control	MK 0003-0112	1 x 1.2 ml

Additional materials and equipment (not supplied in the test kit)

Pre-PCR and sample preparation area:

- RNA extraction Kits:
  - Qiagen - Cat No./ID: 52904 QIAamp Viral RNA Mini Kit (50), Cat No./IDL 52906 QIAmp Viral RNA Mini Kit (250), or
  - Chemagen – Catalog Number: CMG-2015 Prepito Viral DNA-RNA200 Kit (https://chemagen.com/wp-content/uploads/2019/02/CMG-2015-Prepito-Viral-DNA-RNA200-Kit.pdf)
- Nuclease free water
- Mini centrifuge for 0.2 ml and 1.5 ml reaction vessels, EUROIMMUN order no. YG 0612-0101 or similar
- Centrifuge for PCR plates, e.g. VWR, item no. 521-1648 or similar
- Laboratory shaker for reaction vessels (“vortex”), EUROIMMUN order no. YG 0641-0101 or similar
- PCR cooling rack for 0.2 ml reaction vessels, e.g. by Kisker, “IsoFreeze PCR-Rack”, order no. KR-96 or similar
- Pre-PCR rack for 1.5 ml reaction vessels

- Pre-PCR cooling rack for 1.5 ml reaction vessels, e.g. by Kisker, “IsoFreeze Tube-Rack”, order no. KR-20W or similar
- Pipettes (volume-adjustable) and pipette tips with filter, 10, 20, 200 and 1000 µl, DNA and DNase-free
- Reaction vessels 1.5 ml, DNA and DNase-free (recommended: Micro tube 1.5 ml SafeSeal, Sarstedt, item no. 72.706.400)
- Disposable gloves
- PCR reaction vessels:

  For LightCycler® 480 II (Roche):
  LightCycler® 480 Multiwell Plate 96, white, Roche, item no. 04729692001
  For 7500 Fast Real-Time PCR Instrument (Applied Biosystems):
  MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode (0.1 ml), Fisher Scientific (Applied Biosystems), item no. 4346906
  MicroAmp® Fast Optical 96-Well Reaction Plate (0.1 ml), Fisher Scientific (Applied Biosystems), item no. 4346907
  MicroAmp® Optical Adhesive Film, Fisher Scientific (Applied Biosystems), item no. 4311971
  For CFX 96 Touch (Bio-Rad):
  Multiplate™ 96-Well PCR Plates, low profile, unskirted, clear, Bio-Rad, item no. MLL9601
  Microseal 'B' PCR Plate Sealing Film, adhesive, optical, Bio-Rad, item no. MSB1001

Amplification area:

- One of the following real-time PCR cyclers is recommended:
  - 7500 Fast Real-Time PCR Instrument (Applied Biosystems)
  - LightCycler® 480 II (Roche)
  - CFX 96 Touch (Bio-Rad)

Storage and stability

- **PCR Mix A (green cap) and B (yellow cap) SARS-CoV-2:** Ready for use. Protect from light and store in **pre-PCR area** at -18°C to -25°C. Before use, thaw no more than five times at +2°C to +8°C for a short period (do not exceed 60 minutes), mix by multiple inversion (**do not vortex!**) and centrifuge to collect the solutions at the bottom.
- **Positive control SARS-CoV-2 (purple cap):** Ready for use. Store in the sample preparation area at -18°C to -25°C. Before use, thaw no more than five times at +2°C to +8°C for a short period (do not exceed 60 minutes), mix and centrifuge to collect the solution at the bottom.
- **RNA internal control (white cap):** Ready for use. Store in **pre-PCR area** at -18°C to -25°C. Before use, thaw no more than five times at +2°C to +8°C for a short period (do not exceed 60 minutes), mix and centrifuge to collect the solution at the bottom.

In-use stability following the first opening

After opening, the reagents are stable until the indicated expiry date when stored under the specified conditions and protected from contamination, unless stated otherwise herein. **EUROIMMUN recommends thawing the reagents no more than five times and, if required, making aliquots.**

Sample storage and stability

- Store specimens at +2°C to +8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.
- Store RNA samples exclusively in the sample preparation area and according to the recommendations provided in the RNA preparation system. If not tested immediately, or if stored after testing, EUROIMMUN recommends storing the extracted RNA samples at -18°C to -25°C, ideally at -80°C. Short-term storage at a temperature of +4°C to +8°C is generally possible. **Note:** Inappropriate storage of RNA samples as well as frequent thawing and re-freezing can damage the RNA and should be avoided; otherwise false negative results may occur.

- The products that are used for the sample taking, storage and transport of samples should generally render the appropriate sample amount and quality as required for the use in this test system. Depending on the type of sample and the transport medium used, specific storage and, for RNA isolation, pre-treatment of the sample may be required. Please observe the instructions provided by the manufacturer.
- For more information refer, to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens form Persons for Coronavirus Diesease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html and Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

Warnings and precautions

- For use under an Emergency Use Authorization
- *For In Vitro* Diagnostic Use
- For Prescription Use Only
- The EURORealTime SARS-CoV-2 has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.
- The EURORealTime SARS-CoV-2 has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The EURORealTime SARS-CoV-2 is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The test may only be performed by qualified personnel proficient in handling infectious materials and trained in the techniques of real-time PCR and in vitro diagnostic procedures. Recommendations for the performance of molecular amplification tests must be observed [8].
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.
- Please observe the operating instructions with respect to the real-time PCR cyclers.
- If the packed reagents are visibly damaged, do not use the test kit.
- Do not use reagents past their expiration data.
- Before using the product, read the instruction for use carefully. Use only the valid version provided with the product.
- Do not substitute or mix the EUROIMMUN reagents with reagents from other manufacturers.
- Due to the high analytical sensitivity of a PCR, extreme caution should be exercised to ensure the purity of all test kit components and samples. Contamination by the smallest amounts of PCR products can lead to false positive results. To prevent contamination, the individual steps of the test procedure should ideally be carried out in three separate rooms (pre-PCR, sample preparation, amplification area), as described in the following [9]. Store and use all instruments, pipettes, reagents and materials as well as protective equipment (laboratory coat, protective goggles, disposable gloves) only in the room where they belong. Minimize the risk for contamination by:

  Pre-PCR area

  The pre-PCR area is used for storage and handling of PCR reagents. Do not bring samples, PCR products or RNA positive controls into this room.

Sample preparation area

This room is used for isolation of RNA from the samples and for pipetting of extracted samples into the PCR mix. Do not bring PCR products into this room.

Amplification area

PCR amplification in the real-time PCR cycler is carried out in this area. After PCR, the fragments of the target nucleic acid to be detected are present in million- or billionfold amplified copies. Even the smallest carry-over into the pre-PCR and sample preparation rooms via clothing, instruments, materials or aerosols can lead to false results in samples tested thereafter and should be absolutely avoided.

- All work surfaces, devices and tools, e.g. racks, should be cleaned with DNA-/RNA-degrading agents, e.g. diluted hypochlorite solution [10] on a regular basis, ideally after each use. Note: Some disinfectants, e.g. diluted ethanol, are not suited to degrade nucleic acids. Please contact EUROIMMUN for further information on this matter.

Preparation of reagents

Preparation of reagents is not required. All kit components are ready for use.

Waste disposal

Samples, controls and used reaction vessels should be handled as potentially infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Quality control

To ensure correctness of results, parallel testing (extraction and amplification) of a no template negative control (not included) and the positive control of the test kit must be performed in every test run.

Internal control:
In order to check for potential real-time PCR inhibition or extraction failure the RNA internal control (IC) containing an artificial *in vitro* transcribed sequence with no homology to any other known sequence is provided with the kit. The IC must be added to each reaction. Without the IC, negative results must be classified as “invalid”.

Negative control (Not Included):
In order to exclude contamination of the test components with amplifiable nucleic acid a negative control must be performed with each batch of extracted specimens, using a nucleic acid-free solution, i.e., water or TE buffer, as the sample (negative control, NC). If the NC is omitted or classified as “invalid”, all results obtained with this test must be evaluated as “invalid”.
It is mandatory to add the IC to the negative control to control the extraction and reverse transcription process of RNA in samples. Negative controls without amplification signal for the IC are classified as “invalid”. If the IC is used as extraction control, EUROIMMUN recommends using the IC also for the negative control already during RNA preparation.

Positive control:
In order to monitor the RT-PCR reaction and reagents including the RNA detection a SARS-CoV-2 positive control (PC) is needed. The Positive Control consists of in *vitro* transcribed RNA fragments containing the target sequences, is provided with the kit, and must be tested in each RT-PCR run. The positive control must be added directly to the PCR reaction and must not be used for extraction. If the PC is omitted or classified as “invalid”, all results obtained with this test must be classified as “invalid”.

External controls must be tested in accordance laboratory practices and accreditation requirements.

Assay procedure

Overview:

Please read the chapter **"Notes on the test performance and safety"** before starting.

1. RNA preparation	- Prepare RNA according to the instructions provided by the manufacturer of the preparation system used

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2. Real-time PCR	- Choose the cycler-specific temperature profile - Insert position and name of each sample and control - Assign detection filters to each sample and control - Prepare PCR master mix from PCR Mixes A and B - Pipette master mix into PCR plate - Add samples and controls - Incubate in the real-time PCR cycler

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3. Evaluation	- Start evaluation process in the real-time PCR cycler - Check and validate results - Print result documents

Detailed work instructions

Sample material:

For validation/evaluation of the EURORealTime SARS-CoV-2 test with clinical samples, RNA isolated from oropharyngeal swabs was extracted using the QIAamp® Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions for use and the CMG-2015 Prepito Viral DNA/RNA200 Kit (Chemagen) according to the manufacturers' instructions. **When using the CMG-2015 Prepito Viral DNA/RNA200 Kit, however, 300 µl lysis buffer were added to 300 µl sample, deviating from the manufacturer's instructions.**

Preparation of RNA from patient samples:

The QIAamp® Viral RNA Mini Kit (Qiagen) or the CMG-2015 Prepito Viral DNA/RNA200 Kit (Chemagen) provide a sufficient RNA quantity and quality for the use with this test system, provided that validated specimen types are used.

6 µl of the IC must be added to the lysis buffer or the mixture of sample and lysis buffer (it also must be added to the negative control). The IC must not be added directly to the sample material before addition of lysis buffer! If the lysis buffer is spiked with the IC before addition to the samples, the added volume of IC should be 10% of the elution buffer volume needed in a given run. Example: If 10 samples are purified and every sample is eluted in a volume of 60 µl elution buffer, the corresponding volume of lysis buffer needs to be added to 60 µl of IC.

RNA Extraction

Subsequent to the addition of the IC, RNA is extracted according to the instructions of the manufacturer:

- QIAamp Viral RNA Mini Kit (Qiagen): The QIAamp Viral RNA Mini Kit (Qiagen) uses a manual purification according to the manufacturer's instructions with 140 µl specimen input volume and 60 µl elution volume.
- CMG-2015 Prepito Viral DNA/RNA200 Kit (Chemagen). The CMG-2015 Prepito Viral DNA/RNA200 Kit (Chemagen) uses automated purification with the following modified extraction procedure: 300 ul lysis buffer are added to 300 µl specimen input volume. The remaining procedure follows the manufacturer's instructions for use including the elution with 50 µl elution buffer.

Note: EUROIMMUN recommends performing the additional centrifugation step before elution of the RNA according to the manufacturer's specifications to remove any ethanol residue from the purification. Ethanol has a strongly inhibitory effect on the PCR reaction and may lead to false negative or invalid test results

Preparation of run

The purified nucleic acid is reverse transcribed using PCR Mixes A and B (10 µl PCR Master Mix (5 µl Mix A + 5 µl Mix B) + 10 µl purified RNA/negative control or 10 µl of positive control) into cDNA which is then subsequently amplified using the compatible PCR platforms of the 7500 Fast Real-Time PCR Instrument (Applied Biosystems), the LightCycler 480 II (Roche), or the CFX 96 Touch (Bio-Rad).

Real-time PCR cycler programming

The following instrument settings and the temperature profile must be manually implemented in the real-time PCR cycler software:

Instrument Settings

7500 Fast Real-Time PCR Instrument (Applied Biosystems)

Reaction Volume Per Well:	20 µl
Passive reference:	None
Cycler type:	7500 Fast (96 Wells)
Experiment type:	Quantitation-Standard Curve*
Reagent:	TaqMan reagents
Run-time:	Standard (~2 hours to complete run)
Software Setting:	Check the default setting “automatic threshold” and correct it, if required, by adjusting the threshold value; information on this step are provided in the instrument manual.

* Setting required for this instrument even though there is no quantitative result.

LightCycler 480 II (Roche):

Plate type:	White Plates
Detection Format:	Dual Color Hydrolysis Probe
Reaction Volume:	20 µl
Ramp Rate:	Default
Software Setting:	Data interpretation using the “Abs Quant/2nd Derivative Max” Option.

CFX 96 Touch (Bio-Rad):

Sample volume:	20 µl
Plate Type:	BR Clear
Plate Size:	96 Wells
Scan Mode:	All Channels
Software Setting:	Check the default setting “Single Threshold” and correct it, if required, by adjusting the threshold value.

Reporter Dye Setting:

Parameter	LightCycler® 480 II (Roche)	7500 Fast Real-Time PCR Instrument (Applied Biosystems)	CFX 96 Touch (Bio-Rad)
SARS-CoV-2	FAM (465-510)	FAM (Quencher None)	FAM
IC	VIC / HEX / Yellow555 (533-580)	VIC (Quencher None)	HEX

Temperature profile:

	Number cycles	Analysis Mode (only for LightCycler® 480 II)	Acquisition Mode (only for LightCycler® 480 II)	Temperature [°C]	Time [min:sec]
Reverse transcription	1	None	None	45	10:00
Denaturation	1	None	None	95	02:00
Amplification	45	Quantification*	None	95	00:15
			Single	58	00:45
Cooling	1	None	None	37	00:20

*While this is the setting of the instrument there is no quantitation of the template.

Note: The PCR program given and the settings were optimised for the real-time PCR cyclers LightCycler® 480 II (Roche), 7500 Fast Real-Time PCR Instrument (Applied Biosystems) and CFX 96 Touch (Bio-Rad) using the PCR reaction vessels recommended above. The use of other real-time PCR cyclers and other PCR reaction vessels has not been validated.

Creation of a run

Create the run as follows and according to the manufacturer's instructions of the real-time PCR cycler used:

- Enter the name and the position of each sample
- Define the sample type for each sample (positive/negative control or patient sample)
- Apply the detection filters for each sample
- Enter the temperature profile according to the table

Preparation of master mix

The following steps must be performed in the **pre-PCR area** (room 1):

- Prepare the PCR plate
- Remove PCR Mixes A and B and controls from the freezer approx. 15 minutes before pipetting the PCRs and thaw in dedicated area/room at +2°C to +8°C. Keep samples and reagents at +2°C to +8°C throughout the pipetting procedure. Protect from light, do not exceed 60 minutes at +2°C to +8°C!
- After thawing at +2°C to +8°C, mix the PCR Mixes A and B by multiple inversion (**do not vortex!**) and centrifuge to collect the solutions at the bottom.
- Prepare the PCR master mix according to the pipetting scheme shown below. EUROIMMUN recommends always taking into account a surplus of 10% to compensate for pipetting inaccuracies.
- Pipette the indicated amount of PCR Mixes A and B into a 1.5 ml reaction vessel using a new pipette tip each time. Mix thoroughly by pipetting up and down several times.

  The basis for master mix calculation per sample is:

Component	Volume [µl]
PCR Mix A	5
PCR Mix B	5
IC[1]	1
∑ (Total)	11

- Pipette 10 µl of master mix into each well (see plate layout) (one single pipette tip may be used).

Note: Avoid air bubbles during pipetting! Return all kit components to frozen storage at -18°C to -25°C immediately after use.

[1] Only add the IC to the master mix if it has not yet been used during RNA isolation (see **"Preparation of RNA from patient samples"**). If the IC has already been used as sample extraction control, it MUST NOT be added at this stage. Instead, add the corresponding amount of RNA/DNA/nuclease-free water to the master mix.

Addition of samples

The following steps must be performed in the **sample preparation area**:

- If required, thaw the RNA samples and controls, mix by vortexing briefly and centrifuge for a short period to collect the solutions at the bottom.
- Pipette 10 µl of each purified RNA sample/negative control or 10 µl of positive control into the corresponding well containing the master mix. **Change the pipette tip with every step!**
- Seal the wells with care; ensure that the caps or film are placed correctly!
- Centrifuge the PCR plate or strips briefly to collect the solutions at the bottom.

Amplification reaction

The following steps must be performed in the **amplification area**:

- Place the prepared PCR plate into the real-time PCR cycler.
- Start the real-time PCR cycler.

Please verify manually that the correct settings, cycling program and sample assignment have been chosen.

Warning: Heated lid and incubation block of the real-time PCR cycler may reach temperatures of up to +110°C. There is a risk of skin burns. Please observe the operating instructions with respect to the instrument.

Software settings of the used real-time PCR cyclers:

Data analysis is performed by the software of the instrument after any baseline adjustments (if needed, performed per instrument manual). EUROIMMUN recommends the following settings:

Software Settings

7500 Fast Real-Time PCR Instrument (Applied Biosystems)

Check the default setting “automatic threshold” and correct it, if required, by adjusting the threshold value.

LightCycler 480 II (Roche):

Data interpretation using the “Abs Quant/2nd Derivative Max” Option

CFX 96 Touch (Bio-Rad):

Check the default setting “Single Threshold” and correct it, if required, by adjusting the threshold value.

Interpretation of Results

The detection of SARS-CoV-2 RNA is performed using two target regions, which are both detected in the same fluorescence channel (FAM).

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Interpretation of Controls

	SARS-CoV-2 FAM	IC VIC	Result
Positive Control	+ (≤ Ct 33)	-	Valid
	-	-/+	Invalid[1]
Negative Control	-	+	Valid
	-	-	Invalid[1] (no or inhibited[2] amplification signal for internal control)
	+	-/+	Invalid[1] (contamination)

[1]If the negative control and/or the positive control shows an invalid result all test results are to be evaluated as “invalid”.
[2]If the negative control shows no or an inhibited amplification signal for the IC all patient test results are to be evaluated as “invalid”.

Examination and Interpretation of Patient Specimen Results:

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted and all patient specimens need to be retested after a root cause has been identified and eliminated. The detection of SARS-CoV-2 RNA is performed using a total of two target regions, which are both detected in the same fluorescence channel (FAM).

Interpretation of patient samples

	SARS-CoV-2 FAM	IC VIC	Result Interpretation	Action
Patient Sample	-	+	SARS-CoV-2 RNA not detected	Report Result
	-	-	**Invalid** (no amplification signal for internal control)	Invalid Result, specimen needs to be re-tested from re-extraction or re-collection from patient for test.
	+	-/+	SARS-CoV-2 RNA detected	Report Result

General note for evaluation: EUROIMMUN recommends repeating the analysis for all samples showing an ambiguous or atypical curve that does not allow a clear interpretation.

Limitations of the Procedure:

- It is essential to adhere to the instructions provided in this package insert. Modifications of the procedure are not allowed under the Emergency Use Authorization.
- For diagnosis, the clinical symptoms of the patient should always be taken into account along with the obtained results. Interpretation must be performed by personnel trained and experienced with this kind of experiment.
- Users should be trained to perform this assay and competency should be documented.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if:
  - specimens are improperly collected, transported or handled
  - amplification inhibitors are present in the specimen
  - the sample is low positive and contains an amount of pathogenic material below the LoD of this test.
  - Spontaneous mutations within the target sequence are present. While this risk associated with spontaneous mutations in the target sequence is mitigated in the test's design, if failure to detect the target is expected it is recommended to test the specimen with a different test that detects different target sequences from the SARS-CoV-2 genome.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- For diagnosis, the clinical symptoms of the patient should always be taken into account along with the molecular diagnostic results. The result of the PCR analysis must, if necessary, be assessed together with results from other diagnostic methods.
- In molecular biological test procedures, further very rare sequence variants which are not yet included in the sources used for specificity and sensitivity analysis of primers and probes and which may affect the test result can generally not be excluded.
- Real-time PCR is used to detect the genetic material of a pathogen. This detection method is not necessarily synonymous with the presence of intact pathogens in the sample that are able to reproduce. Under certain circumstances, a pathogen's genetic material can still be detected after successful treatment.
- A device performance out of specifications and deviations from the described test procedure, specified storage conditions, materials, devices and/or recommended sample material may lead to deviations from the results that are obtained when all instructions are followed. The internal and external controls can help to detect errors. They may, however, not detect every possible error. Each laboratory should validate their modifications and ensure that the device specifications are met.

Conditions of Authorization for the Laboratory:

The EURORealTime SARS-CoV-2 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/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

However, to assist clinical laboratories using the EURORealTime SARS-CoV-2 (referred to as "your product" in the conditions below), the relevant Conditions of Authorization are listed below and are required to be met by laboratories performing the EUA test:

A. Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

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

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

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

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

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

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

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

Performance Characteristics

Limit of Detection (LoD) – Analytical Sensitivity:

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive.

First, a tentative LoD was determined by testing 7 serial dilutions prepared by spiking recombinant virus containing SARS-CoV-2 RNA (Seracare, AccuPlex SARS-CoV-2 Reference Material; 5000 copies/ml) into oropharyngeal swab matrix negative for SARS-CoV-2. Each dilution was tested with 3 individual extraction replicates. The tentative LoD was determined to be 150 copies/mL.

The tentative LoD was confirmed by testing 21 replicates of negative oropharyngeal swab matrix spiked independently with the Accuplex reference material and extracted with the Chemagen CMG-2015 Prepito Viral DNA/RNA200 Kit and the Qiagen QIAamp Viral RNA Mini Kit. Replicates were tested on the Roche LightCycler 480 II. The final LoD for both extraction methods was determine to the 150 copies/mL/

The LoD of 150 cp/ml was then verified for the Applied Biosystems 7500 Fast Real-Time PCR and Bio-Rad CFX 96 Touch cyclers using the same procedure described above. The LoD was confirmed by testing 21 extraction replicates for each extraction kit (CMG-2015 Prepito Viral DNA/RNA200 Kit and QiAmp Viral RNA Mini Kit).

LoD Confirmation in Oropharyngeal Swab Specimens

Instrument	Valid Replicates	SARS-CoV-2		IC		SARS-CoV-2 RNA Detection Rate
		n	Mean Ct	n	Mean Ct	
QIAamp® Viral RNA Mini Kit (Qiagen)						
Roche LightCycler 480 II	21	21	37.24	21	29.82	100.0%
Applied Biosystems 7500 Fast	21	21	37.27	21	29.63	100.0%
Bio-Rad CFX 96 Touch	21	20	36.57	21	28.48	95.2%
CMG-2015 Prepito Viral DNA/RNA200 Kit (Chemagen)						
Roche LightCycler 480 II	21	21	36.18	21	29.30	100.0%
Applied Biosystems 7500 Fast	21	21	35.14	21	29.02	100.0%
Bio-Rad CFX 96 Touch	21	21	34.94	21	27.85	100.0%

Inclusivity – Analytical Sensitivity:
The target genes are N and ORF1ab. An alignment was performed with the oligonucleotide primer and probe sequences with all publicly available complete nucleic acid sequences for SARS-CoV-2 in GenBank as of April 30, 2020 (n = 1523 ORF1ab gene and 1534 N gene; search term: “SARS-CoV-2”) to demonstrate the predicted inclusivity of the EURORealTime SARS-CoV-2. All alignments show 100% identity of the panel (at least one of the target detection systems of this test) to the available SARS-CoV-2 sequences.

The risk of false negative results due to mismatches is mitigated in the dual target design of the test. The risk associated with the mismatched N-gene sequences is considered insignificant as the virus strains associated with these sequences showed 100% sequence match to the ORF1ab primer/probe system, ensuring the EURORealTime SARS-CoV-2 would still report a “SARS-CoV-2 RNA Detected” result. The mismatches with the N-gene detection system are only at the very 5’ end of the forward primer, and it is possible that the primer and therefore the N-gene primer/probe detections system may still be detected by the N-gene in a real-world setting. In the 1534 sequences in the databased only one sequence (accession number MT325588) was detected for which detection could be compromised due to mismatches in both targets (single mismatch in the middle of the ORF1ab probe binding site and three mismatches in the 5’ region of the N-Forward Primer).

Cross-Reactivity – Analytical Specificity: Cross-reactivity of the EURORealTime SARS-CoV-2 was evaluated using both *in silico* analysis and wet testing against normal and pathogenic organisms found in the respiratory tract.

in silico analysis:

BLASTn analysis queries of the EURORealTime SARS-CoV-2 primers and probes were performed against the corresponding reference sequence indicated in the table (GenBank Acc#). The database "refseq_representative_genomes" was used. The search was restricted to the corresponding organism e.g. Human coronavirus (strain SARS) (taxid:694009). The other search parameters were set to default.

In summary, no organisms (except for SARS-coronavirus) exhibited >80% homology to the forward primer, reverse primer, and probe for either the ORF1ab or N target.

For SARS-coronavirus (NC_004718.3), only one primer for ORF1ab and N target shows *in-silico* cross-reactivity with SARS-CoV. This is not sufficient to produce an unspecific signal in the EURORealTime SARS-CoV-2. Furthermore, "wet-lab" cross-reactivity/interference studies were performed with SARS-coronavirus (see below) and no cross-reactivity was seen using the EURORealTime SARS-CoV-2.

In Silico Cross-Reactivity: EURORealTime SARS-CoV-2

No.	Pathogen	GenBank Acc#	%Homology Test **Forward Primer**	% Homology Test **Reverse Primer**	%Homology Test **Probe**
1	Human coronavirus 229E	NC_002645.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
2	Human coronavirus OC43	NC_006213.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
3	Human coronavirus HKU1	NC_006577.2	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
4	Human coronavirus NL63	NC_005831.2	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
5	SARS-coronavirus	NC_004718.3	N specific forward primer: 91 %	No significant similarity (<80%)	ORF1ab specific reverse Primer: 96%
6	MERS-coronavirus	NC_019843.3	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
7	Adenovirus (e.g. C1 Ad. 71)	NC_001405.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
8	Human Metapneumovirus (hMPV)	NC_039199.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
9	Parainfluenza virus 1-4	NC_003461.1; NC_003443.1; NC_001796.2; NC_021928.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
10	Influenza A & B	NC_026437.1; NC_002204.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
11	Enterovirus (e.g. EV68)	NC_038308.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
12	Respiratory syncytial virus	NC_001803.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
13	Rhinovirus	NC_038311.1; NC_001490.1; NC_038312.1; NC_001617.1; NC_038878.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
14	*Chlamydia pneumoniae*	NC_000922.1	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
15	*Haemophilus influenzae*	taxid:727	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
16	*Legionella pneumophila*	taxid:446	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
17	*Mycobacterium tuberculosis*	taxid:1773	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
18	*Streptococcus pneumoniae*	taxid:1313	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
19	*Streptococcus pyogenes*	taxid:1314	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
20	*Bordetella pertussis*	taxid:520	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
21	*Mycoplasma pneumoniae*	taxid:2104	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
22	Pneumocystis jirovecii (PJP)	taxid:42068	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)

No.	Pathogen	GenBank Acc#	%Homology Test Forward Primer	% Homology Test Reverse Primer	%Homology Test Probe
23	*Candida albicans*	taxid:5476	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
24	*Pseudomonas aeruginosa*	taxid:287	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
25	*Staphylococcus epidermis*	taxid:1282	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)
26	*Streptococcus salivarius*	taxid:1304	No significant similarity (<80%)	No significant similarity (<80%)	No significant similarity (<80%)

Cross-reactivity wet-testing:

Wet-testing against normal and pathogenic organisms of the respiratory tract was performed to confirm the results of the *in silico* analysis. The DNA/RNA of each organism identified below were spiked with 1 ng/reaction, if available, and was tested in duplicate with the ***EURORealTime SARS-CoV-2***. No cross-reactive results were detected.

Wet-Testing Cross-Reactivity: EURORealTime SARS-CoV-2

No.	Virus/Bacteria/Parasite	Sample type (Source)	Purified Nucleic Acid Amount	Result
1	Influenza A H1	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV-2 RNA Not Detected
2	Influenza B*	Culture Material (Zeptometrix)	4 µl/reaction	SARS-CoV-2 RNA Not Detected
3	Respiratory Syncytial Virus (subtype A)	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV-2 RNA Not Detected
4	Respiratory Syncytial Virus (subtype B)*	Culture Material (Zeptometrix)	<1ng/µl	SARS-CoV-2 RNA Not Detected
5	human Parainfluenza 1	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV-2 RNA Not Detected
6	Parainfluenza 2	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV-2 RNA Not Detected
7	Parainfluenza 3	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV-2 RNA Not Detected
8	Parainfluenza 4*	Culture Material (Zeptometrix)	<1ng/µl	SARS-CoV-2 RNA Not Detected
9	Rhinovirus	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV-2 RNA Not Detected
10	Enterovirus 71*	Culture Material (EUROIMMUN)	<1ng/µl	SARS-CoV-2 RNA Not Detected
11	Coronavirus NL63	Genomic RNA (Erasmus MC, Viroscience[2])	1 ng/reaction	SARS-CoV-2 RNA Not Detected
12	Coronavirus MERS	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV-2 RNA Not Detected
13	Coronavirus OC43	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV-2 RNA Not Detected
14	Coronavirus SARS HKU39849	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV-2 RNA Not Detected
15	Coronavirus 229E	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV-2 RNA Not Detected
16	Coronavirus HKU1	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV-2 RNA Not Detected
17	*Legionella pneumophila*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV-2 RNA Not Detected
18	*Mycoplasma pneumoniae*	Genomic DNA (UKSH)[3]	1 ng/reaction	SARS-CoV-2 RNA Not Detected
19	Adenovirus 5*	Cell Culture (EUROIMMUN)	<1ng/µl	SARS-CoV-2 RNA Not Detected
20	*Chlamydia pneumoniae*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV-2 RNA Not Detected
21	*Haemophilus influenza*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV-2 RNA Not Detected
22	*Mycobacterium tuberculosis**	Genomic DNA (Vircell – Spain)	<1ng/µl	SARS-CoV-2 RNA Not Detected
23	*Streptococcus pneumoniae*	Genomic DNA (UKSH)[3]	1 ng/reaction	SARS-CoV-2 RNA Not Detected
24	*Streptococcus pyogenes*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV-2 RNA Not Detected
25	*Bordetella pertussis* DSM-5571	Genomic DNA (UKSH)[3]	1 ng/reaction	SARS-CoV-2 RNA Not Detected

No.	Virus/Bacteria/Parasite	Sample type (Source)	Purified Nucleic Acid Amount	Result
26	*Pneumocystis jirovecii**	Control Material (Qnostics)	<1ng/µl	SARS-CoV-2 RNA Not Detected
27	Human Genomic DNA (from buffy coat)	Genomic DNA (UKSH)[3]	100 ng/reaction	SARS-CoV-2 RNA Not Detected
28	Human Genomic RNA	EUROIMMUN	100 ng/reaction	SARS-CoV-2 RNA Not Detected

[1]Leibiniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (Germany)
[2]The Department of Viroscience of the Erasmus MC (Netherlands)
[3] UKSH [University Medical Center Schleswig-Holstein, Institute of Medical Microbiology and Hygiene (Lübeck, Germany)]
*Extraction of nucleic acid from the microorganism was attempted either from cell culture material (Adenovirus 5, Enterovirus 71, Influenza B, Parainfluenza 4 virus, and Pneumocystis jirovecii) or from commercially available genomic RNA (Mycobacterium tuberculosis, Vendor Vircell) or from control material (RSV B, Qnostics) but provided concentrations of <1ng/µl. Therefore, 4µl of undiluted nuceic acid solutions were used for testing.

Microbial Interference Studies – Analytical Specificity: It was further shown that microorganisms and viruses that can live in the respiratory tract or that are closely related to the pathogen to be detected, as well as human nucleic acids do not interfere with SARS-CoV-2 detection. For this purpose, SARS-CoV-2 detection was confirmed using SARS-CoV-2 *in vitro* transcripts for the ORF1ab- and N-gene at 3 copies/µL (30 copies per reaction) in the presence of genomic nucleic acids of the organisms and viruses in the table below. All DNAs and RNAs of the organisms or viruses were spiked with 1 ng/reaction, if available. To exclude interferences with human genomic DNA or RNA, 100 ng of each per reaction were tested.

Microbial Interference: EURORealTime SARS-CoV-2

No.	Virus/Bacteria/Parasite	Source/ Sample type	Purified Nucleic Acid Amount	Result
1	Influenza A H1	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV2 RNA Detected
2	Influenza B*	Culture Material (Zeptometrix)	4 µl/reaction	SARS-CoV2 RNA Detected
3	Respiratory Syncytial Virus (subtype A)	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV2 RNA Detected
4	Respiratory Syncytial Virus (subtype B)*	Culture Material (Zeptometrix)	4 µl/reaction	SARS-CoV2 RNA Detected
5	human Parainfluenza 1	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV2 RNA Detected
6	Parainfluenza 2	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV2 RNA Detected
7	Parainfluenza 3	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV2 RNA Detected
8	Parainfluenza 4*	Culture Material (Zeptometrix)	4 µl/reaction	SARS-CoV2 RNA Detected
9	Rhinovirus	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV2 RNA Detected
10	Enterovirus 71*	Culture Material (EUROIMMUN)	4 µl/reaction	SARS-CoV2 RNA Detected
11	Coronavirus NL63	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV2 RNA Detected
12	Coronavirus MERS	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV2 RNA Detected
13	Coronavirus OC43	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV2 RNA Detected
14	Coronavirus SARS HKU39849	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV2 RNA Detected
15	Coronavirus 229E	Genomic RNA (Viroscience[2])	1 ng/reaction	SARS-CoV2 RNA Detected
16	Coronavirus HKU1	Culture Material (Zeptometrix)	1 ng/reaction	SARS-CoV2 RNA Detected
17	*Legionella pneumophila*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV2 RNA Detected
18	*Mycoplasma pneumoniae*	Genomic DNA (UKSH)[3]	1 ng/reaction	SARS-CoV2 RNA Detected
19	Adenovirus 5*	Cell Culture (EUROIMMUN)	4 µl/reaction	SARS-CoV2 RNA Detected
20	*Chlamydia pneumoniae*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV2 RNA Detected
21	*Haemophilus influenza*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV2 RNA Detected
22	*Mycobacterium tuberculosis**	Genomic DNA (Vircell – Spain)	4 µl/reaction	SARS-CoV2 RNA Detected
23	*Streptococcus pneumoniae*	Genomic DNA (UKSH)[3]	1 ng/reaction	SARS-CoV2 RNA Detected

No.	Virus/Bacteria/Parasite	Source/ Sample type	Purified Nucleic Acid Amount	Result
24	*Streptococcus pyogenes*	Genomic DNA (DSMZ)[1]	1 ng/reaction	SARS-CoV2 RNA Detected
25	*Bordetella pertussis*	Genomic DNA (UKSH)[3]	1 ng/reaction	SARS-CoV2 RNA Detected
26	*Pneumocystis jirovecii**	Control Material (Qnostics)	4 µl/reaction	SARS-CoV2 RNA Detected
27	Human Genomic DNA (from buffy coat)	Genomic DNA (UKSH)[3]	100 ng/reaction	SARS-CoV2 RNA Detected
28	Human Genomic RNA	EUROIMMUN	100 ng/reaction	SARS-CoV2 RNA Detected

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*Extraction of nucleic acid from the microorganism was attempted either from cell culture material (Adenovirus 5, Enterovirus 71, Influenza B, Parainfluenza 4 virus, and Pneumocystis jirovecii) or from commercially available genomic RNA (Mycobacterium tuberculosis, Vendor Vircell) or from control material (RSV B, Qnostics) but provided concentrations of <1ng/µl. Therefore, 4µl of undiluted nuceic acid solutions were used for testing.

Endogenous Interference Substances Studies – Analytical Specificity: The influence of the following substances was investigated by adding them to BAL matrix or viral transport medium before RNA extraction using the QIAamp® Viral RNA Mini Kit (the concentration given in brackets refers to the sample to be extracted): 2% lidocaine solution (5%), 20% benzocaine solution (5%), 0.2% chlorhexidine gluconate solution (10%), 100,000 I.U./ml nystatin solution (10%), guaifenesin (5 mg/ml), nasal spray (active ingredient: xylometazoline, 10%), saline (0.9%, 3.5%), EDTA blood (5%), 20 mg/g miconazole gel (10%), and acyclovir cream (10%). After proper purification using the QIAamp® Viral RNA Mini Kit, none of these substances produced an inhibitory effect on the SARS-CoV-2 detection in samples with ORF1ab and N-gene *in vitro* transcripts at 3 copies/µL (30 copies per reaction).

Endogenous Interference Substances: EURORealTime SARS-CoV-2

No.	Potential Interfering Substance	Concentration	Replicate 1 Result	Replicate 2 Result
1	2% lidocaine solution	5%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
2	20% benzocaine solution	5%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
3	0.2% chlorhexidine gluconate solution	10%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
4	100,000 I.U./ml nystatin solution 10%	1%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
5	Guaifenesin	5 mg/ml	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
6	Commercially Available Nasal Spray with Xylometazolinhydrochlorid 0,1%	10%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
7	Saline (physiologic)	0.9%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
8	Saline (hypertonic)	3.5%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
9	EDTA blood	5%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
10	20 mg/g Miconazole gel	10%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected
11	Acyclovir Cream	10%	SARS-CoV-2 RNA Detected	SARS-CoV-2 RNA Detected

Clinical Performance

Clinical performance of the EURORealTime SARS-CoV-2 test was evaluated by testing clinical oropharyngeal swab samples and comparing the results of the EURORealTime SARS-CoV-2 test to the prior results of a well-validated SARS-CoV-2 molecular comparator test.

A total of 80 oropharyngeal swab samples (50 positives; 30 negatives) were extracted using the QIAamp Viral RNA Mini Kit (Qiagen; 55 samples) and the CMG-2015 Prepito Viral DNA/RNA200 Kit (Chemagen; 25 samples). The QIAamp extraction was performed according to the manufacturers' instructions. The CMG-2015 Prepito Viral DNA/RNA200 Kit extraction was used with the same modified procedure validated for use with the EURORealTime SARS-CoV-2 test (per detailed work instructions above).

Testing of the clinical sample panel with the EURORealTime SARS-CoV-2 was done at EUROIMMUN (Dassow, Germany) on the LightCylcer480 II.

Clinical Performance of the EURORealTime SARS-CoV-2 with Oropharyngeal Swab Specimens

N = 80 Oropharyngeal swabs		Comparator	
		Positive	Negative
EURORealTime SARS-CoV-2	Positive	48	0
	Negative	2	30

Positive Agreement 96.0% (48/50) **95**% **C.I.:** 86.3% - 99.5%
Negative Agreement 100.0% (30/30) **95**% **C.I.:** 88.4% - 100.0%

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Technical support

In case of technical problems you can obtain assistance via the EUROIMMUN website (www.euroimmun.us/contact) and by contacting technical support directly via phone at 973-794-4241 or by email at support@euroimmun.us.

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
PCR MIX A	PCR Mix A		Storage temperature
PCR MIX B	PCR Mix B		Unopened usable until (YYYY-MM-DD)
POS CONTROL	Positive control		Manufacturing date (YYYY-MM-DD)
INT CONTROL	Internal control		Manufacturer
IVD	In vitro diagnostic medical device		Observe instructions for use
LOT	Lot description	CE	CE-labelled
	Protect from sunlight		

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