rTEST COVID-19 qPCR kit Rapid / 400 reactions

User Information

Ver. 1.2 (en), 12/2021

NOTICE!
This kit is intended for *in vitro* diagnostic purposes.

1. Explanations and abbreviations

Baseline: basic background fluorescence or so-called „noise"
BHQ-1: Black Hole Quencher-1, a non-fluorescent quencher for the FAM fluorescent reporter dye
BHQ-2: Black Hole Quencher-2, a non-fluorescent quencher for the YY fluorescent reporter dye
BHQ-3: Black Hole Quencher-3, a non-fluorescent quencher for the Cy5 fluorescent reporter dye
BSL: bio-safety level
COVID-19: disease caused by the SARS-CoV-2 virus (COronaVIrus Disease 2019)
Ct: threshold cycle, the cycle in which the fluorescence signal of the reaction exceeds the set fluorescence threshold
Cy5: cyanine-derived fluorescent reporter dye in the red region
DNA: deoxyribonucleic acid
E gene: a gene encoding the small membrane envelope protein of the SARS-CoV-2 virus
FAM: 6-carboxyfluorescein, a fluorescent reporter dye
LoD: limit of detection
NTC: No Template Control
PC: Positive Control
RdRP gene: a gene encoding the RNA-dependent RNA polymerase of the SARS-CoV-2 virus
RFU: relative fluorescence unit
RNA: ribonucleic acid
RNase P gene: a gene encoding human nuclear ribonuclease P
rTEST: room TEmperature STable
RT-qPCR: reverse transcription qPCR
qPCR: quantitative Polymerase Chain Reaction
SARS-CoV-2: Severe Acute Respiratory Syndrome CoronaVirus 2
Threshold: the point at which the fluorescence reporter signal significantly exceeds background fluorescence
YY: Yakima Yellow®, a fluorescent reporter dye developed by Epoch Biosciences

2. Intended use

The rTEST COVID-19 qPCR Rapid kit is a qualitative *in vitro* diagnostic test designed to detect the presence of SARS-CoV-2 genetic material in biological samples obtained by gargling without RNA extraction. The kit is intended exclusively for use in a diagnostic laboratory with the appropriate equipment, safety standards, and properly trained personnel.

3. Test principle

The rTEST COVID-19 qPCR Rapid kit is among the first fully room temperature stable diagnostic kits to detect genomic RNA of SARS-CoV-2.
It can be stored at room temperature for two weeks enabling transportation without dry ice and easier handling of the kit upon arrival. The room temperature stability is allowed by lyophilized mixes of SARS-CoV-2-specific primers/probes, internal positive control (full genomic RNA of SARS-CoV-2 virus spiked with human RNA), and proprietary, room temperature stable 1-step RT-qPCR reagents from Solis Biodyne.
The rTEST COVID-19 qPCR Rapid kit is an innovative, improved, and re-designed version of the WHO-recommended Charité, Berlin protocol. The kit contains three sets of primers and hydrolysis probes (TaqMan®) in a single multiplexed reaction targeting the SARS-CoV-2 specific E and RdRP genes together with human RNase P. TaqMan probes for E, RdRP, and RNase P genes are conjugated to YY, FAM, and Cy5, respectively. This enables multiplexed detection of SARS-CoV-2 and human RNase P as an internal control to validate proper sample collection and performance of the test. **For E and RdRP genes, we developed proprietary dual TaqMan® probes to increase the sensitivity and specificity of our 1-Step RT-qPCR method.** The full genomic RNA of SARS-CoV-2 virus spiked with human RNA provided by the Biomedical Center of the Slovak Academy of Sciences is included as an internal positive control.

The RT and qPCR reactions are conducted in a one tube, one-step fashion.

One package of the kit is sufficient for 400 tests. Multiplexing allows co-detection of SARS-CoV-2 (confirmation of positive COVID-19) and human RNase P, which can be used for secondary confirmation of negative samples as well as to validate sample collection.

NOTICE!

- **Read all instructions in this user manual before starting work.**

CAUTION!

SARS-CoV-2 is a dangerous pathogen so follow all applicable regulations and recommendations for BSL2+ or BSL3 class laboratories.

4. Kit composition

- **1x lyophilized mix of SARS-CoV-2_E, SARS-CoV-2_RdRP and human RNase P primers and probes labelled as E gene/RdRP gene/RNase P Mix (must be dissolved in 800 µl of PCR water)**
- **1x lyophilized PC BMC5 spiked with human RNA (must be dissolved in 320 µl of PCR water)**
- **2x 800 µl 5X One-step Probe Direct Mix 3**
- **1x 200 µl 40X One-step RT Direct Mix 2**
- **1x lyophilized 10X Rapid Lysis Buffer A**
- **1x 4.4 ml 10X Rapid Lysis Buffer B**
- **2x 5 ml PCR water**
- **1x Instruction for use**

5. Storage and shelf life

All kit components can be transported and stored at room temperature (15-25 °C) up to two weeks. The kit can be routinely stored at –20 °C. The shelf life of the kit is a maximum of 12 months from the date of manufacture. The exact expiration date of the kit is indicated on the outer box. The exact expiration date of the individual components of the kit is indicated on the inner packaging/vials.

CAUTION!

Do not use the kit after the expiration date, which is stated on the outer box.

The kit and its individual components are designed to perform 400 reactions.
For users who perform fewer reactions within one run, we recommend that you aliquot all kit components according to internal procedures and the standard number of reactions per run. Aliquoting the kit components minimizes the need to reopen individual tubes and thus minimizes the risk of contamination of the kit components. Aliquoting the components of the kit will also prevent repeated thawing and freezing of the individual components, which may lead to reduced efficiency. Before aliquoting, it is necessary to completely thaw the individual components of the kit. Thawing is optimally performed gently, by incubation in a refrigerator (at 4 °C) for approx. 2 hours. Immediately before aliquoting, it is necessary to mix the contents of the tubes thoroughly, but at the same time carefully, avoiding the formation of bubbles, until the mixture is completely homogeneous. In the case of tubes containing enzymes (5X One-step Probe Direct Mix 3, 40X One-step RT Direct Mix 2), we recommend mixing by rotating the tube several times, pulse vortexing, or short vortexing for no longer than 5 seconds without generating any bubbles. Pipette the enzymes carefully and slowly; otherwise, the viscosity of the buffer may lead to pipetting errors.

NOTICE!

- **Wear suitable protective clothing, gloves, and eye/face protection.**
- **Never pipette by mouth.**
- **Never eat, drink, or smoke in the laboratory and do not use any cosmetics.**
- **Always wash your hands thoroughly when handling specimens and reagents.**

6. Consumables and equipment not included in the kit

- Real-time PCR equipment: rTEST COVID-19 qPCR Rapid diagnostic kit has been validated and tested on Agilent devices - Mx3005P® and AriaMx®, Thermo Fisher Scientific - QuantStudio™ 5, BioRad - CFX96™, Analytik Jena - qTOWER^3
- Laminar flow box
- Vortex

- Mini centrifuge
- Centrifuge with rotor for plates
- Personal protective equipment: powder-free disposable laboratory gloves, goggles, protective shield, FFP3 respirator, protective clothing
- Laboratory plasticware certified sterile and free of DNA, RNA, DNases and RNases: reagent tubes, PCR tubes, PCR strips, PCR plates, PCR foils, sterile pipette tips with filter
- Autoclavable adjustable micropipettes
- Bio-waste container
- Autoclavable test tube racks
- Cooling PCR tube/plate holder

7. Workflow

CAUTION!

Any work with the kit must be performed by qualified personnel.

NOTICE!

- **Workspaces must be arranged in such a way that there are separate, dedicated rooms (zones) for each step in the workflow: nucleic acid isolation, preparation of amplification reactions, or amplification and detection of amplification products. The amplified products must never come into contact with space intended for the isolation of nucleic acids or for the preparation of amplification mixtures.**
- **Within each room (zone), maintain separate, dedicated laboratory equipment, consumables, and personal protective equipment (lab coats, gloves, etc.) for each step in the workflow. Never use the same laboratory equipment, consumables, and personal protective equipment in different rooms (zones).**
- **Always handle all biological samples as potentially infectious material and avoid direct contact with biological material. Avoid spilling samples and reagents and generating aerosols.**
- **After sample preparation, it is advised to avoid excessive delay before starting the reaction in a thermocycler.**
- **Follow the enclosed instructions for use thoroughly.**

7.1. Sampling and sample preparation

rTEST COVID-19 qPCR Rapid kit is designed to be used with direct gargle samples. Using sample types other than described below, improper sample collection, transport, and storage procedures may lead to impaired functional characteristics of the assay!

NOTICE!

- **The gargling procedure described below is only a recommendation and matches the instructions given to patients during sample collection for clinical validation. The optimal method of gargling for the detection of SARS-CoV-2 has not been determined.**
- **It is NOT advisable to eat, drink, brush teeth, rinse mouth, gargle, chew gum or smoke/vape at least one hour before collecting a sample.**
- **Commercial saline solutions are recommended; however, in-house prepared solutions are acceptable as long as the water source used is WFI (Water for Injection) quality or equivalent.**

Sample collection:

- The patient is given a suitable container (e.g., polypropylene 50 ml tube) containing 5 ml of isotonic saline solution (0.9% NaCl by weight in water).
- The patient is then asked to gargle with the provided saline solution 10 times for 5 seconds each taking care not to swallow the solution. This takes around 1 minute in total.
- The patient then slowly and carefully spews out the gargle into a prepared container (for example the same one saline was provided in). Once the container is disinfected from the outside, the gargle sample is ready for further processing.
- If inactivation cannot be performed immediately, store the fresh gargle samples at 4 °C forup to a week.

Preparation of 10X Rapid Lysis Buffer A

Add 4180 µl of 10X Rapid Lysis Buffer B to the tube containing lyophilized 10X Rapid Lysis Buffer A. Thoroughly vortex the contents of the tube. Prepare aliquots of the 10X Rapid Lysis Buffer A and store them at -20 °C.

Sample processing:

- Carefully vortex the gargle sample to resuspend any settled material. Aggressive vortexing may lead to foaming and splashing which carries a significant biohazard risk at this point as the sample has not been inactivated yet.
- In a tube with a minimal volume of 200 µl (for example a PCR or 0.5 ml tube) mix gargle (9 parts) with the provided 10X Lysis Buffer A (1 part), specifically:
  90 µl gargle + 10 µl 10X Rapid Lysis Buffer A
- Close the tube and gently mix.
- Let incubate at room temperature for 3 minutes. Modestly longer incubation times (up to 10 minutes) are not detrimental.
- Heat the sample at 95 °C for 7 minutes. This step thermally inactivates viral particles and releases viral RNA into solution.
- Let the sample cool down for a few seconds. Then briefly centrifuge the sample on a tabletop mini centrifuge for 1 minute. Precipitated proteins and other debris will form a pellet at the bottom. The resulting supernatant is to be used as the input for the RT-qPCR.
- Ideally, inactivated gargle samples should be assayed as soon as possible; however, they are stable for up to a day at –20 °C. For long-term, samples should be stored at –70 °C or below. Avoid repeated thawing and freezing of samples.

CAUTION!

- **Do not open different samples at the same time to avoid possible cross contamination.**
- **Vortex and centrifuge the samples in a laminar flow box to prevent aerosol contamination.**
- **Use pipettes designated exclusively for handling specimens and use disposable filter tips that are certified sterile and free of DNA, RNA, DNases and RNases.**

7.2. RT-qPCR

The routine rTEST COVID-19 qPCR Rapid kit workflow is a simultaneous screening & confirmation test to determine the presence of viral RNA for the E and RdRP gene. In addition, having an internal control assay for human RNase P (in Cy5 channel) in multiplexed mode with E and RdRP gene assesses appropriate sample collection and the presence of human RNA within the sample, while eliminating false-negative results.

NOTICE!

- **RNase P is a human transcript, therefore the primers and probe mix designed for RNase P can detect both RNA and genomic DNA if present in the sample.**

7.3. Workspace preparation

Before starting the protocol, first clean the working space of the laminar flow box and adjacent surfaces with a 10% solution of sodium hypochlorite (bleach) and then with a 70% solution of ethanol to remove residual bleach. Use the UV decontamination cycle before and after working in the laminar flow box.

7.4. Preparation of reagents

Remove the necessary kit components from the freezer and thaw completely in the refrigerator (4 °C), on ice, or in a refrigerated tube cooling rack. Once thawed, mix their contents thoroughly and gently until completely homogeneous.
We recommend mixing tubes containing enzymes (5X One-step Probe Direct Mix 3, 40X One-step RT Direct Mix 2) by rotating the tube several times, pulse vortexing, or short vortexing for no longer than 5 seconds without generating any bubbles. Then centrifuge the tubes briefly to remove droplets from the cap and ensure all liquid is at the bottom of the tubes. Pipette enzyme mixes carefully and slowly as the viscosity of the buffer may lead to pipetting errors.
Mixtures of primers and TaqMan® probes designated SARS-CoV-2_E, SARS-CoV-2_RdRP, and Human RNase P (provided as E gene/RdRP gene/RNase P Mix) as well as the PC BMC5 spiked with human RNA are supplied in lyophilized form to increase stability. It is therefore necessary to dissolve mixtures of primers and probes and the positive control in PCR water when the kit is used for the first time.

CAUTION!

Since the colored oligo pellet can become dislodged during shipping, it is crucial to briefly centrifuge every tube before opening. Failure to do so could result in yield loss, because oligo pellets that are not at the bottom of the tube could fly out of the tube when the cap is opened.

Add 800 µl of water from the PCR Water tube to the tubes containing E gene/RdRP gene/ RNase P Mix (400 reactions). In addition, add 320 µl of PCR water to the tube containing lyophilized PC BMC5.

Thoroughly vortex the contents of the tubes. Then briefly centrifuge the tubes to remove droplets from the cap and ensure all liquid is at the bottom of the tubes.

Prepare the reaction mixture as soon as possible after mixing the contents of the individual components of the kit. If necessary, vortex and centrifuge the contents of the tubes once more just before preparing the reaction mixture.
After use, store kit components in the freezer (–20 °C). Avoid repeated thawing and freezing of kit components. If any kit components will be reused within 2 hours, store at 4 °C.

CAUTION!

Always handle reagents in a laminar flow box. Always prepare reagents for amplification separately, preparing reagents exclusively for one analysis at a time. Use pipettes designed exclusively for preparation of reagents and use disposable filter tips. The tips used must be certified sterile and free of DNA, RNA, DNases and RNases.

NOTICE!

- **Use only reagents contained in this kit and reagents recommended by the manufacturer.**
- **Do not combine or mix reagents from different lots.**
- **Do not combine reagents from kits from different manufacturers.**

7.5. Preparation of the reaction mixture

The recommended total volume of one reaction is 20 µl. To prepare the reaction mixture, the individual components of the kit must be mixed in the following order and ratio:

NOTICE!

- **The reaction mixture has limited stability, use it as soon as possible after preparation. If the reaction mixture cannot be used immediately, store it in a refrigerator at 4 °C.**
- **When preparing multiple reactions, it is recommended to make 5 - 10% extra reaction mixture to account for pipetting errors.**

Reaction mixture setup

Kit component	Component volume per reaction
PCR water	5.5 µl
5X One-step Probe Direct Mix 3	4 µl
40X One-step RT Direct Mix 2	0.5 µl
E gene/RdRP gene/RNase P Mix	2 µl
Total volume	**12 µl**

Table of calculated volumes for a given number of reactions:

Kit components	1	2	3	4	5	6	7	8	9	10	96
PCR water	5.5 µl	11 µl	16.5 µl	22 µl	27.5 µl	33 µl	38.5 µl	44 µl	49.5 µl	55 µl	528 µl
5X One-step Probe Direct Mix 3	4 µl	8 µl	12 µl	16 µl	20 µl	24 µl	28 µl	32 µl	36 µl	40 µl	384 µl
40X One-step RT Direct Mix 2	0.5 µl	1 µl	1.5 µl	2 µl	2.5 µl	3 µl	3.5 µl	4 µl	4.5 µl	5 µl	48 µl
E gene/RdRP gene/ RNase P Mix	2 µl	4 µl	6 µl	8 µl	10 µl	12 µl	14 µl	16 µl	18 µl	20 µl	192 µl
Total volume	**12 µl**	**24 µl**	**36 µl**	**48 µl**	**60 µl**	**72 µl**	**84 µl**	**96 µl**	**108 µl**	**120 µl**	**1152 µl**

7.6. Plate preparation and inspection

Add 8 µl of sample to each prepared 12 µl reaction mixture, resulting in a 20 µl total reaction volume.

Prepare the required number of clean PCR tubes, PCR strips, or PCR plates and place them in a refrigerated cooling rack. Mix the prepared reaction mixture thoroughly but at the same time gently by turning the tube several times, pulse vortexing, or short vortexing for no longer than 5 seconds without generating any bubbles. Then centrifuge it briefly to remove droplets from the cap and ensure all the liquid is at the bottom of the tube. Pipette 12 µl of the prepared reaction mixture into individual PCR tubes or wells of a PCR plate in accordance with the required number and position of reactions. Pipette the reaction mixture carefully and slowly as the viscosity of the buffer may lead to pipetting errors. Then transfer the PCR tubes or PCR plate with the pipetted reaction mixture from the laminar flow box for preparation of the reaction mixture to the laminar flow box for finalization of plate preparation. Add 8 µl of sample, or 8 µl of positive control (PC BMC5), or 8 µl of PCR water into appropriate PCR tubes or PCR plate wells. Then tightly seal the individual PCR tubes with the lids or wells of the PCR plate with optical foil. Centrifuge the PCR tubes or PCR plate briefly so that all fluid is at the bottom of the tubes/wells and insert into the real-time PCR instrument.

A minimum of one negative control must be included in each analysis to verify the presence of contamination. A no template control (NTC) containing PCR water is used as a negative control instead of an unknown sample.
A minimum of one positive control (PC) must be included in each analysis to validate the workflow of the analysis and the functionality of the kit components. A reaction containing the positive control (PC BMC5) is used instead of an unknown sample.

The PC BMC5 consists of isolated genomic RNA of SARS-CoV-2 spiked with human RNA.
The PC BMC5 will yield a positive result with all primer and probe sets (e.g., E, RdRP, and RNase P genes).

7.7. Real-time PCR instrument settings

Follow the instructions below to set the assay conditions for the reaction volume, temperature conditions, and optical channels.

Reaction volume:
- **20 µl**

Thermocycling conditions:
- **Reverse transcription: 50 °C, 15 min**
- **Initial denaturation: 95 °C, 10 min**
- **Cycling, 45 cycles:**
  - **» Denaturation: 95 °C, 1 s**
  - **» Annealing/extension: 60 °C, 5 s**

Optical channels used:

- Optical channel for FAM label: blue or green channel according to the real-time PCR device - excitation maximum 495 nm, emission maximum 520 nm
- Optical channel for YY label: yellow channel (HEX, JOE or VIC) according to the real-time PCR device - excitation maximum 525 nm, emission maximum 550 nm
- Optical channel for Cy5 label: red channel - excitation maximum 650 nm, emission maximum 670 nm

Follow the real-time PCR equipment manufacturer's manual and your internal procedures for this type of assay when setting the analysis conditions for the number and type of samples, the distribution of samples on the plate, and the type of plasticware used (tubes, strips, plates).

CAUTION!

Do not modify or change the recommended protocols for PCR analyses.

CAUTION!

Handle amplification products with extreme care to avoid dispersal into the laboratory area and possible contamination of new test specimens. Use pipettes designated exclusively for handling amplification products and use disposable filter tips that are certified sterile and free of DNA, RNA, DNases, and RNases.

7.8. Analysis of the obtained data

To set the baseline and threshold for each reaction, follow the manufacturer's manual for the real-time PCR instrument in accordance with your internal procedures for this type of assay.

8. Interpretation of results

8.1. Interpretation of results and reporting (clinical samples)

- **Sampling and Positive Control Results and Interpretation**
- **No Template Control (NTC)**
  The NTC consists of using nuclease-free water (PCR water) in the RT-qPCR reactions instead of RNA. The NTC reactions for all primer andprobe sets should not exhibit fluorescence amplification curves that cross the threshold line. If any of the NTC reactions exhibit an amplification curve that crosses the cycle threshold line, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.
- **SARS-CoV-2 Positive Control (PC BMC5)**
  The PC BMC5 consists of lyophilized isolated genomic RNA of SARS-CoV-2 spiked with co-precipitant (such as salmon sperm DNA or Baker's yeast tRNA to increase stability) and with human RNA. The PC BMC5 will yield a positive result with all primer and probe sets (i.e., E, RdRP, and RNase P genes). Standard Ct values for the PC BMC5 positive control should exhibit a Ct lower than 35.00 for all E, RdRP, and RNase P genes. The signal level should also result in values above 1000 RFU in all three genes to be considered valid. Indications of an error or failure in the workflow or analysis of the experiment include: the complete absence of a signal, the presence of an amplified signal but with higher Ct values than usual for a given control material, or the presence of a low-level signal. In the case of a negative result in the positive control, it is not possible to unambiguously determine the correctness of other positive/negative results obtained in the given analysis and to distinguish between negative and false negative results. Therefore, the output of such an analysis cannot be evaluated.
- **RNase P (Sampling Control)**
  - » All clinical samples should exhibit fluorescence amplification curves in the RNase P channel that cross the threshold line within 35.00 cycles (Ct < 35.00), thus indicating the presence of the human RNase P gene in the RNA sample. Failure to detect RNase P in any clinical specimens may indicate:
    - Improper handling/processing/storage of clinical materials resulting in loss of RNA and/or RNA degradation.
    - Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
    - Improper assay set up and execution.
    - Reagent or equipment malfunction.
  - » If the RNase P assay does not produce a positive result for human clinical specimens, interpret as follows:
    - If the SARS-CoV-2 E and RdRP assays are positive even in the absence of a positive RNase P, the result should be considered valid. It is possible that some samples may fail to exhibit RNase P amplification curves due to low cell numbers in the original clinical sample. A negative RNase P signal does not preclude the presence of SARS-CoV-2 viral RNA in a clinical specimen.
    - If all SARS-CoV-2 markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the test. If all markers remain negative after re-testing, report the results as invalid and collect a new specimen if possible.
- **SARS-CoV-2 markers (E and RdRP)**
  - » When all controls exhibit the expected performance, a specimen is considered negative if the amplification curves for both SARS-CoV-2 genes (E, RdRP) DO NOT cross the threshold line within 40.00 cycles (Ct > 40.00) AND the RNase P amplification curve DOES cross the threshold line within 35.00 cycles (Ct < 35.00).
  - » When all controls exhibit the expected performance, a specimen is considered positive if the amplification curves for both SARS-CoV-2 genes (E, RdRP) cross the threshold line within 40.00 cycles (Ct < 40.00). The RNase P may or may not be positive as described above, but the SARS-CoV-2 result is still valid.
  - » When all controls exhibit the expected performance and the amplification curves for both SARS-CoV-2 genes (E, RdRP) AND the RNase P marker DO NOT cross the cycle threshold within 40.00 cycles (Ct < 40.00) AND 35.00 cycles (Ct < 35.00), respectively, the result is considered invalid. The sample should be re-tested. If the re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.
  - » When all controls exhibit the expected performance and the amplification curve for one of the SARS-CoV-2 specific genes (E or RdRP but not both) crosses the threshold line within 40.00 cycles (Ct < 40.00) the result is considered inconclusive. The sample should be re-tested. If the same result is obtained, report the inconclusive result. Consult with your public health authority, as appropriate, to request guidance and/or to coordinate transfer of the specimen for additional analysis.
- **rTEST COVID-19 qPCR Rapid diagnostic test results interpretation guide**
  The table below lists the expected results for rTEST COVID-19 qPCR Rapid test diagnostic panel. If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please consult your public health authority.

Expected performance of controls included in the Rapid rTEST COVID-19 qPCR kit

Control Type	External Control Name	SARS-CoV-2 E	SARS-CoV-2 RdRP	Human RNase P	Expected Ct values	Possible causes of the unexpected results
Positive	PC BMC5	+	+	+	Ct < 35.00	Substantial reagent failure including primer and probe integrity or degradation of positive control
Negative	NTC	–	–	–	None detected	Reagent and/ or environmental contamination

Deviation from the expected performance of the controls suggests improper assay set up and/or execution, or failure/malfunction of reagents and/or equipment could have occurred. Invalidate the run and re-test.

Interpretation of SARS-CoV-2 test results and corresponding actions

SARS-CoV-2 E	SARS-CoV-2 RdRP	Human RNase P	Result Interpretation[a]	Report	Actions
+	+	+/–	SARS-CoV-2 detected	Positive SARS-CoV-2	Report results to sender.
+	–	+/–	Inconclusive Result	Inconclusive	Repeat testing. If the repeated result remains inconclusive, contact your public health authorities for further instructions or guidance.
–	+	+/–			
–	–	+	SARS-CoV-2 not detected	Not detected	Report results to sender. Consider testing for other respiratory viruses[b].
–	–	–	Invalid result	Invalid	Repeat testing. If the repeated result remains invalid, consider collecting a new specimen from the patient.

[a] Laboratories should report their diagnostic results as appropriate and in compliance with their specific reporting system.

[b] Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest COVID-19 while diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If SARS-CoV-2 infection is still suspected, re-testing should be considered in consultation with public health authorities.

NOTICE!

- **The rTEST COVID-19 qPCR Rapid diagnostic kit is designed for use by qualified and trained laboratory personnel with sufficient experience in real-time RT-qPCR testing techniques.**

9. Functional characteristics

9.1. Limit of Detection (LoD)

Evaluation of analytical sensitivity (limit of detection) was performed on a multiplexed reaction targeting the SARS-CoV-2 E and RdRP genes as well as human RNase P. The test was performed using the positive control „SARS-Related Coronavirus 2 (SARS-CoV-2) Stock" (ZeptoMetrix, https://www.zeptometrix.com/products/nattrolsars-related-coronavirus-2-sars-cov-2-stock), which in the undiluted state contains 1000 copies of template per 1 µl. Dilutions were prepared by serial dilutions of the „SARS-Related Coronavirus 2 (SARS-CoV-2) Stock" in SARS-CoV-2 negative patient sample obtained by gargling, resulting in samples with concentrations of 40 copies/µl, 10 copies/µl, 4 copies/µl, 2 copies/µl, 1 copy/µl and 0.5 copies/µl of sample obtained by gargling that were used in the analytical sensitivity test. The assay was performed in 8 replicates for each prepared dilution. The test confirmed the high sensitivity of the rTEST COVID-19 qPCR Rapid diagnostic kit. Reliable template detection of both E and RdRP genes was demonstrated down to 1 copy/µl of sample, which was also confirmed in an extensive LoD experiment where 24 out of 24 replicates were positive for E gene and 23 for RdRP gene.

Limit of detection of SARS-CoV-2 E and RdRP tests

	E gene/RdRP gene/RNase P gene		
	Total number of replicates	Number of reactions with positive results (E/RdRP/RNase P)	Detection success (%, E/RdRP/RNase P)
40 copies/µl of sample	8	8/8/8	100/100/100
10 copies/µl of sample	8	8/8/8	100/100/100
4 copies/µl of sample	8	8/8/8	100/100/100
2 copies/µl of sample	8	8/8/8	100/100/100
1 copy/µl of sample*	8	8/8/8	100/100/100
0.5 copies/µl of sample	8	8/8/8	100/100/100
1 copy/µl of sample#	24	24/23/24	100/96/100

* After considering the absence of RNA extraction comparable with rTEST COVID-19 qPCR Allplex
# Extensive LoD

9.2. Test specificity

Evaluation of specificity (cross-reactivity to other coronaviruses and respiratory viruses) was performed for both SARS-CoV-2 E and RdRP genes. The test was performed using the control material „Coronavirus RNA specificity panel" (EVAg, European Virus Archive - Global), which contains RNA viruses HCoV-229E, HCoV-OC43, HCoV-NI63, SARS-CoV HKU39849, and MERS-CoV, each in a separate tube.
The EDX SARS-CoV-2 Standard (Exact Diagnostics) was used as a reference material for evaluation of specificity. A set of respiratory viruses (Vircell) containing RNA of Influenza A H1N1, Novel Influenza A H1N1, Influenza A H3N2, Influenza A H5N1, Novel Influenza B, Human parainfluenza, Respiratory syncytial virus and Human rhinovirus, each provided in a separate tube, were used to assess cross-reactivity to respiratory viruses. All assays were performed in triplicate for each of the indicated viruses.

The test confirmed the high specificity of the rTEST COVID-19 qPCR Rapid diagnostic kit. A positive result was recorded exclusively in reactions containing SARS-CoV-2 RNA (Exact Diagnostics, PC BMC5) in the presence of primers/probe sets for the E and RdRP genes. The occurrence of contamination by synthetic positive controls in various commercially available products used to perform RT-qPCR diagnosis of COVID-19, e.g., primers, probes, or RT-qPCR mixtures is a global problem. The mixture of primers and probes for SARS-CoV-2_E in the rTEST COVID-19 qPCR Rapid kit was designed not to amplify the most commonly used synthetic positive controls at all or only with low efficiency. Thus, the rTEST COVID-19 qPCR Rapid kit can also be used effectively in workplaces that have a problem with contamination when testing for the presence of the SARS-CoV-2 E gene.

Evaluation of chemical stability (e.g., degradation of probes during thermocycling) was performed for both SARS-CoV-2 E and RdRP assays. The test was performed as an analysis of multiple no template controls (NTC), including 20 replicates for the E gene and 40 replicates for the RdRP gene.

The assay confirmed the high chemical stability of the oligonucleotides contained in the rTEST COVID-19 qPCR Rapid kit. In each of the analyzed NTCs, a negative result was recorded with no indication of an increase in signal or the presence of amplification.

9.3. Clinical performance evaluation

Evaluation of the clinical performance of the rTEST COVID-19 qPCR Rapid kit was performed on a multiplexed reaction targeting the SARS-CoV-2 E and RdRP genes as well as human RNase P. The evaluation was performed on a selected set of 105 positive and 94 negative clinical samples obtained by gargling, which were confirmed by a reference method used for routine testing by regional public health authorities of the Slovak Republic. Samples were exposed to one freeze-thaw cycle before virus inactivation (rTEST COVID-19 qPCR Rapid kit) and RNA extraction (reference method), respectively. Testing of this selected set of samples was performed with blinded samples.

The analysis uniformly confirmed the results of the reference method for all samples evaluated and demonstrated the high reliability and reproducibility of the results obtained with the rTEST COVID-19 qPCR Rapid kit *(see table below)*.
Based on the data obtained, the rTEST COVID-19 qPCR Rapid kit has 100% diagnostic sensitivity and 100% diagnostic specificity. The results of RNase P gene detection showed high homogeneity of the analyzed samples and also confirmed the suitability of this assay as an internal control for collection and RNA extraction from a clinical sample.

Clinical performance of rTEST COVID-19 qPCR Rapid kit

	Reference method			rTEST COVID-19 qPCR Rapid		
	E gene	RdRP gene	RNase P gene	E gene	RdRP gene	RNase P gene
Number of correctly identified positive samples	105	105	105	105	105	105
Number of false positive samples	0	0	0	0	0	0
Number of correctly identified negative samples	94	94	94	94	94	94
Number of false negative samples	0	0	0	0	0	0

10. Disposal

NOTICE!

- **Decontaminate any material that has come into contact with biological samples with 3% sodium hypochlorite for a minimum of 30 minutes or autoclave at 121 °C for a minimum of 60 minutes before disposing.**
- **All used equipment, tips, tubes, work materials, and protective clothing should be considered potentially contaminated and disposed of in accordance with applicable infectious waste disposal regulations.**
- **Dispose of remaining reagents and material in accordance with applicable safety regulations.**

11. Troubleshooting and safety reporting
(Medical device vigilance)

In case of any problems contact:

MultiplexDX, s. r. o.
Manufacturer

Address: Ilkovičova 8
841 04 Bratislava

Tel.: +421 2 902 68 310
Email: rtest@multiplexdx.com

12. Symbols

	Manufacturer
LOT	Batch number
-20°C	Recommended storage temperature
Σ	Package size
CE	This product complies with the requirements of European Directive 98/79/EC on *in vitro* diagnostic medical devices
	Date of manufacturing
IVD	*In vitro* diagnostic medical devices
	Attention, follow the safety instructions in the operating instructions that came with this product

CE IVD Registration code: P1430A

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