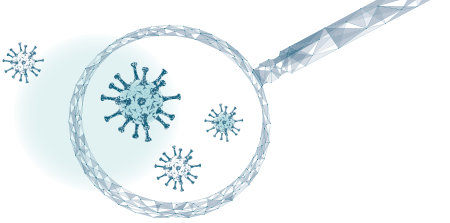
rTEST COVID-19 qPCR B.1.1.7 kit / 200 tests

User Information

Ver. 1.0 (en), 01/2021



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

1. Explanations and abbreviations

B.1.1.7: a novel SARS-CoV-2 lineage characterized by distinct mutations and increased transmissibility
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-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 5, a fluorescent reporter dye
DNA: deoxyribonucleic acid
FAM: 6-carboxyfluorescein, a fluorescent reporter dye
LoD: Limit of Detection
ND: Not Detected
NTC: No Template Control
PC: Positive Control
qPCR: quantitative Polymerase Chain Reaction
RFU: relative fluorescence unit
RNA: ribonucleic acid
RNase P gene: a gene encoding human nuclear ribonuclease P
ROX: 6-carboxy-X-rhodamine, a fluorescent reference dye used to normalize the reporter dye signal
RT-qPCR: reverse transcription qPCR
rTEST: room TEmperature STable
S gene: a gene encoding the spike protein of the SARS-CoV-2 virus
SARS-CoV-2: Severe Acute Respiratory Syndrome CoronaVirus 2
Threshold: the point at which the fluorescence reporter signal significantly exceeds background fluorescence

2. Intended use

The rTEST COVID-19 qPCR B.1.1.7 kit is a qualitative in vitro diagnostic test designed for specific detection of genetic material specific to the new SARS-CoV-2 lineage B.1.1.7 in biological samples obtained primarily from the human upper airways (nose and nasopharynx). This variant first described by the United Kingdom is associated with higher viral loads and increased transmissibility (i.e., more efficient and rapid transmission). 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 B.1.1.7 kit is the first fully room temperature stable kit to detect genomic RNA of SARS-CoV-2 lineage B.1.1.7. It can be stored at room temperature for at least one month 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 S gene and SARS-CoV-2 lineage B.1.1.7 specific primers/probes, internal positive control (full genomic viral RNA of SARS-CoV-2 lineage B.1.1.7 spiked with human RNA), and proprietary, room temperature stable 1-step RT-qPCR reagents from Solis Biodyne.

The rTEST COVID-19 qPCR B.1.1.7 kit is a newly designed differential test distinguishing between consensus SARS-CoV-2 and the new B.1.1.7 variant. The kit contains two sets of primers and hydrolysis probes (TaqMan®) targeting either the consensus (C95) sequence of the SARS-CoV-2 S gene or the B.1.1.7 variant, which encompasses two lineages defining deletions HV69-70 and Y144. Both sets allow the detection of the human RNase P gene. The TaqMan® probes for the S gene of both the consensus SARS-CoV-2 and B.1.1.7 variant are conjugated to FAM, while the TaqMan® probe for RNase P is conjugated to Cy5. This enables multiplexed detection of either the consensus SARS-CoV-2 S gene or B.1.1.7 variant, and human RNase P, which serves as an internal control to validate proper sample collection, RNA extraction, and performance of the test. **For both variants of S gene, we developed proprietary dual TaqMan® probes to increase the sensitivity and specificity of our 1-Step RT-qPCR method.** The full genomic viral RNA of SARS-CoV-2 lineage B.1.1.7 spiked with human RNA provided by the Biomedical Center of the Slovak Academy of Sciences is included as an internal positive control. The 5X One-step Probe CoV Mix (ROX) reagent includes a ROX passive reference dye as an inert additive providing a constant fluorescent signal for sample normalization in the real-time PCR assay. Due to the unique molecular structure of the supplied ROX dye, the solution is compatible with all ROX-dependent (both high-ROX and low-ROX) and ROX-independent real-time PCR cyclers.

The RT and qPCR reactions are conducted in a one tube, 1-step fashion. The rTEST COVID-19 qPCR B.1.1.7 kit is an additional confirmatory kit used for closer characterization of SARS-CoV-2 positive samples, to confirm or exclude the B.1.1.7 variant. Patient samples that tested positive for SARS-CoV-2 in the primary test are tested for the presence of the consensus or B.1.1.7 variant of S gene. Multiplexing allows co-detection of either consensus SARS-CoV-2 S gene or B.1.1.7 variant with human RNase P, which can be used for secondary confirmation of negative samples, as well as to validate sample collection, RNA extraction, and performance of the qPCR reaction.

One package of the kit is sufficient for 400 testing reactions and is intended to detect the B.1.1.7 variant in patient samples that previously tested SARS-CoV-2 positive in the primary test. It is recommended to run two parallel reactions to detect the presence of consensus SARS-CoV-2 S gene in one reaction and B.1.1.7 variant in another one. The human RNase P internal positive control assay is multiplexed with both the consensus SARS-CoV-2 S gene and B.1.1.7 variant, serving to verify the quality of the swab and RNA extraction, to determine the presence of human RNA within the extracted sample, while eliminating false negative results.

NOTICE!

- **Read all instructions in this user manual, the outer packaging, and the component labels 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 primers/probes mix for SARS-CoV-2_S gene and human RNase P labelled as S gene/ RNase P Mix (must be dissolved in 400 µl of PCR water)**
- **1x lyophilized primers/probes mix for SARS-CoV-2_S gene B.1.1.7 variant and human RNase P labelled as B.1.1.7/RNase P Mix (must be dissolved in 400 µl of PCR water)**
- **1x lyophilized PC B.1.1.7 (must be dissolved in 100 µl of PCR water)**
- **2x 800 µl 5X One-step Probe CoV Mix (ROX)**
- **1x 200 µl 40X One-step SOLIScript® CoV Mix**
- **2x 5 ml PCR water**
- **1x Instructions for use**

5. Storage and shelf life

All kit components can be transported and stored at room temperature (15-25 °C) up to 1 month. The kit can be routinely stored at –20 °C. The 5X One-step Probe CoV Mix (ROX) contains ROX as a reference dye that is photo-sensitive and should be protected from light whenever possible. 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 CoV Mix (ROX), 40X One-step SOLIScript® CoV

Mix), 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: the rTEST COVID-19 qPCR B.1.1.7 kit has been validated and tested on Agilent devices - Mx3005P® and AriaMx®, Thermo Fisher Scientific - QuantStudio™ 5, BioRad - CFX96™, Analytik Jena - qTOWER3
- Laminar flow box
- Vortex mixer
- 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
- PCR tube/plate cooler

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), laboratory equipment, and consumables for each step in the workflow: nucleic acid isolation, preparation of amplification reactions, and amplification and detection of amplification products. The amplified products must never come into contact with space, equipment, and consumables intended for the isolation of nucleic acids or for the preparation of amplification mixtures.**
- **Use separate, designated lab coats, gloves, and all other personal protective equipment for each step in the workflow. Never use the same 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 RNA extraction

Improper sample collection, transport, and storage procedures as well as RNA extraction may result in incorrect test results. Users should refer to established guidelines for collecting, transporting, and storing samples and should adhere to manufacturer instructions for specimen collection. Sterile swabs with a plastic or aluminum shaft and a synthetic swab must be used for sampling. Swabs with a wooden shaft and / or a cotton swab must not be used. Following sample collection, immerse swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

RNA extraction from the samples should be performed promptly after collection according to the manufacturer's instructions. If a delay in extraction is expected, samples must be stored at 4 °C for up to 12 hours after collection or at –70 °C for long-term sample storage. Avoid repeated thawing and freezing of samples.

The rTEST COVID-19 qPCR B.1.1.7 kit was validated and tested on RNA samples obtained using the following extraction kits:

- Zymo Research, Quick-RNA Viral 96 Kit (Catalog # R1040, R1041)
- Cytiva (formerly GE Healthcare Life Sciences), RNAspin 96 Kit (Catalog # 25050075)
- RNAdvance Viral Genomic Reagent (magnetic beads, Catalog # C59543, C63510)

CAUTION!

During the workflow, always wear personal protective equipment and work in a laminar flow box. Viral RNA can also cause infection. Thus, handle all samples with caution and treat as potentially infectious material.

NOTICE!

- **The samples analyzed are intended solely for this type of analysis.**
- **Strictly follow sample processing guidelines to avoid degradation of nucleic acids.**
- **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 rTEST COVID-19 qPCR B.1.1.7 kit workflow consists of testing samples that were previously identified to be positive for SARS-CoV-2 by a primary screening test in order to confirm the presence of the consensus SARS-CoV-2 S gene and/or B.1.1.7 variant. It is mandatory to run two parallel multiplex confirmatory tests to determine the presence of consensus SARS-CoV-2 S gene and B.1.1.7 variant. Moreover, an internal control test for human RNase P is included in both assays with consensus SARS-CoV-2 S gene or B.1.1.7 variant to confirm swab and RNA extraction efficiency and assay performance.

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 CoV Mix (ROX), 40X One-step SOLIScript® CoV Mix) 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_S, SARS-CoV-2_B.1.1.7, and Human RNase P (provided as S gene/RNase P Mix or B.1.1.7/RNase P Mix) as well as the PC B.1.1.7 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 400 µl of water from the PCR Water tube to the tube labeled as S gene/RNase P Mix (200 reactions) and 400 µl of water from the PCR water tube to the tube labeled as B.1.1.7/RNase P Mix (200 reactions). In addition, add 100 µl of water from the PCR water tube to the tube containing positive control PC B.1.1.7.

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 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:

Table 1. **Reaction mixture setup**

Kit component	Component volume per reaction	
	S gene/RNase P	B.1.1.7/RNase P
PCR water	8.5 µl	8.5 µl
5X One-step Probe CoV Mix (ROX)	4 µl	4 µl
40X One-step SOLIScript® CoV Mix	0.5 µl	0.5 µl
S gene/RNase P Mix **or** B.1.1.7/RNase P Mix	2 µl	2 µl
Total volume	**15 µl**	**15 µl**

Table 2.
Calculated volumes for a given number of reactions

Number of reactions	1	2	3	4	5	6	7	8	9	10	96
PCR water	8.5 µl	17 µl	25.5 µl	34 µl	42.5 µl	51 µl	59.5 µl	68 µl	76.5 µl	85 µl	816 µl
5X One-step Probe CoV Mix (ROX)	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 SOLIScript® CoV Mix	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
S gene/RNase P Mix **or** B.1.1.7/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	**15 µl**	**30 µl**	**45 µl**	**60 µl**	**75 µl**	**90 µl**	**105 µl**	**120 µl**	**135 µl**	**150 µl**	**1440 µl**

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.**

NOTICE!

- **The reactions with S gene/RNase P Mix and B.1.1.7/RNase P Mix must be run side by side on the same PCR plate (or PCR tubes in one PCR run) to assure the homogeneity of the results!**

7.6. Plate preparation and inspection

Add 5 µl of sample to each prepared 15 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 15 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 5 µl of sample, or 5 µl of positive control (PC B.1.1.7), or 5 µl of PCR water (NTC) 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 separate NTC for each set of primers/probes (S gene/RNase P, B.1.1.7/RNase P) tested must be included.

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 B.1.1.7) is used instead of an unknown sample. A separate PC for each set of primers/probes (S gene/RNase P, B.1.1.7/RNase P) tested must be included.

The PC B.1.1.7 consists of isolated viral genomic RNA of SARS-CoV-2 variant B.1.1.7 spiked with human RNA. The PC B.1.1.7 will yield a positive result with all primer and probe sets (SARS-CoV-2 primers/probes for consensus S gene and B.1.1.7 variant, and primers/probe for human RNase P).

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: 55 °C, 10 min**
- **Initial denaturation: 95 °C, 10 min**
- **Cycling, 45 cycles:**
  - **» Denaturation: 95 °C, 15 s**
  - **» Annealing/extension: 60 °C, 30 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 ROX dye: orange or red channel according to the real-time PCR device - excitation maximum 575 nm, emission maximum 605 nm
- Optical channel for Cy5 label: red channel - excitation maximum 650 nm, emission maximum 670 nm

If the real-time PCR device supports normalization to the ROX passive reference dye, we recommend performing the analysis with this function enabled. The 5X One-step Probe CoV Mix (ROX) contains ROX with a unique molecular structure as a reference dye providing a constant fluorescent signal for sample normalization of the assay. The fluorescent signal of the ROX dye does not interfere with the detection channel of the FAM and Cy5 fluorescent label. As a result, the rTEST COVID-19 qPCR B.1.1.7 kit is fully compatible with all ROX-dependent and ROX-independent real-time PCR cyclers.

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)

- **Extraction 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 and probe 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.

- **Positive control (PC B.1.1.7)**
  The PC B.1.1.7 consists of lyophilized isolated genomic RNA of SARS-CoV-2 lineage B.1.1.7 spiked with human RNA and co-precipitant (such as salmon sperm DNA or Baker's yeast tRNA to increase stability). The PC B.1.1.7 will yield a positive result with all primer and probe sets (i.e., SARS-CoV-2 S gene, B.1.1.7 variant, and RNase P gene). Standard Ct values for the PC B.1.1.7 positive control should exhibit a Ct lower than 35.00 for all tested markers. The signal level (i.e., the relative fluorescence without normalization with the ROX dye) should also result in values above 1000 RFU 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 (Extraction Control)**
  - » All clinical samples should exhibit fluorescence amplification curves in the RNase P reaction 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 extraction of nucleic acid from 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 B.1.1.7 **and/or** SARS-CoV-2 S 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 in a clinical specimen.
    - — If SARS-CoV-2 S **and** RNase P assays are negative for the specimen, the result should be considered **invalid** for the specimen. If residual specimen is available, repeat the RNA extraction procedure and repeat the test. If all markers remain negative after re-testing, report the results as invalid and a new specimen should be collected if possible.

- **SARS-CoV-2 markers (consensus S gene and B.1.1.7 variant)**
  - » When all controls exhibit the expected performance, the sample is considered **B.1.1.7 negative** if the amplification curve for B.1.1.7 primers/probe mix does not exceed the threshold line within 40.00 cycles (Ct > 40.00) or if it exceeds the threshold line by **twenty or more** cycles later when compared to the amplification curve for the consensus SARS-CoV-2 S gene. The RNase P amplification curve may or may not be positive and cross the threshold line within 35.00 cycles (Ct < 35.00).
  - » When all controls exhibit the expected performance, the sample is considered **B.1.1.7 negative** if the amplification curve for B.1.1.7 primers/probe mix exceeds the threshold line by **eight** cycles later compared to the amplification curve for the consensus SARS-CoV-2 S gene. This profile indicates **the presence of a SARS-CoV-2 variant containing the HV 69-70 deletion**. The RNase P may or may not be positive as described above, but the result is still valid.
  - » When all controls exhibit the expected performance, a specimen is considered **B.1.1.7 positive** if the amplification curve for B.1.1.7 primers/probes mix exceeds the threshold line within 40.00 cycles (Ct < 40.00) and no later than **five** cycles compared to the amplification curves for the consensus SARS-CoV-2 S gene. The RNase P may or may not be positive as described above, but the result is still valid.
  - » When all controls exhibit the expected performance and the amplification curves for both SARS-CoV-2 S genes (consensus and B.1.1.7. variant) **and** the RNase P marker **do not** cross the threshold line within 40.00 cycles (Ct < 40.00) **and** 35.00 cycles (Ct < 35.00), respectively, the result is considered **invalid**. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for SARS-CoV-2 S gene 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 only the SARS-CoV-2 B.1.1.7 variant (but not consensus SARS-CoV-2 S gene) crosses the threshold line within 40.00 cycles (Ct < 40.00) the result is considered **inconclusive**. The extracted RNA should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. 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.

Table 3.
Expected performance of controls included in the rTEST COVID-19 qPCR B.1.1.7 kit

Control Type	External Control Name	SARS-CoV-2 S gene	B.1.1.7	Human RNase P	Expected Ct values	Possible causes of the unexpected results
Positive	PC B.1.1.7	+	+	+	Ct < 35.00	Substantial reagent failure including primer and probe integrity
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.

- **rTEST COVID-19 qPCR B.1.1.7 test results interpretation guide**
  The table below lists the expected results for the rTEST COVID-19 qPCR B.1.1.7 diagnostic test. 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.

NOTE: To set the fluorescence threshold, we recommend following the manual included with the thermal cycler used or the CDC recommendations.

NOTICE!

- **Before using the rTEST COVID-19 qPCR B.1.1.7 kit, we recommend that you calibrate the real-time**

PCR instrument. Follow the user´s instructions of your real-time PCR instrument.

- The rTEST COVID-19 qPCR B.1.1.7 kit is designed for use by qualified and trained laboratory personnel with sufficient experience in real-time RT-qPCR testing techniques.

Table 4. **Interpretation of SARS-CoV-2 test results and corresponding actions**

SARS-CoV-2 S gene	Difference in Ct values between SARS-CoV-2 B.1.1.7 and S gene[b]	Human RNase P	Result Interpretation[a]	Report	Actions
+	Max 5 Ct	+/ND	SARS-CoV-2 B.1.1.7 detected	SARS-CoV-2 B.1.1.7 positive	Report the results to sender.
+	Min 8 Ct	+/ND	SARS-CoV-2 HV 69-70 deletion detected	SARS-CoV-2 B.1.1.7 negative	Report the results to sender.
+	Min 20 Ct	+/ND	other lineage of SARS-CoV-2 detected	SARS-CoV-2 B.1.1.7 negative	Report the results to sender.
+	ND	+/ND	consensus SARS-CoV-2 detected	SARS-CoV-2 B.1.1.7 negative	Report the results to sender.
ND	+	+/ND	Inconclusive result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat RT-qPCR. If the repeated result remains inconclusive, contact your public health authorities for further instructions or guidance.
ND	ND	ND	Invalid result	Invalid	Repeat extraction and RT-qPCR. 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. ND – Not Detected

[b] The difference in Ct values should be calculated as Ct B.1.1.7 - Ct SARS-CoV-2 S gene

9. Functional characteristics

9.1. Limit of detection

Evaluation of analytical sensitivity (detection limit) was performed on the combinations of primers/probes used for multiplexed detection of SARS-CoV-2_S/RNase P and SARS-CoV-2_B.1.1.7/RNase P. The test was performed using RNA isolated from a patient sample infected with the B.1.1.7 variant of SARS-CoV-2 as confirmed by sequencing. This RNA was diluted to 200 copies of template per 1 µl.
Serial dilutions of the stock standard were prepared resulting in samples with concentrations of 8 copies/µl (= 40 copies/reaction), 2 copies/µl (= 10 copies/reaction), 0.8 copies/µl (= 4 copies/reaction), 0.4 copies/µl (= 2 copies/reaction) and 0.2 copies/µl (= 1 copy/reaction) that were used in the analytical sensitivity test. A synthetic matrix "SARS-CoV-2 Negative" (Exact Diagnostics) containing genomic DNA at a concentration of 75,000 copies/ml was used to dilute the control material. The assay was performed in 8 replicates for each prepared dilution.
The test confirmed the high sensitivity of the rTEST COVID-19 qPCR B.1.1.7 kit. Reliable template detection of SARS-CoV-2 B.1.1.7 variant was demonstrated down to 10 copies per reaction (2 copies/µl) and 2 copies per reaction (0,4 copies/µl) for consensus SARS-CoV-2 S gene.

Table 5. **Limit of detection of SARS-CoV-2 variant B.1.1.7 tests**

	S gene/RNase P gene			B.1.1.7/RNase P gene		
	Total number of replicates	Number of reactions with positive results	Detection success	Total number of replicates	Number of reactions with positive results	Detection success
40 copies/reaction	8	8/8	100/100	8	8/8	100/100
10 copies/reaction	8	8/8	100/100	8	8/8	100/100
4 copies/reaction	8	8/8	100/100	8	5/8	62.5/100
2 copies/reaction	8	8/8	100/100	8	3/8	37.5/100
1 copy/reaction	8	2/8	25/100	8	0/8	0/100

9.2. Clinical performance evaluation

Evaluation of the clinical performance of the rTEST COVID-19 qPCR B.1.1.7 kit was performed for the consensus SARS-CoV-2 S gene as well as the SARS-CoV-2 variant B.1.1.7. The evaluation was performed on a selected set of 65 SARS-CoV-2 positive clinical samples and sequencing revealed 37 of these samples belonging to the B.1.1.7 variant lineage. The rTEST COVID-19 qPCR B.1.1.7 kit correctly identified 36 of the B.1.1.7 variant samples, while one sample was inconclusive.

Testing of this selected set of samples was performed blinded in an external laboratory that is a member of the External Quality Assessment (EQA) scheme organized by institutions such as the European Centre of Disease Prevention and Control (ECDC), Institute of Virology, Charité, Berlin, Germany, and National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.

The clinical evaluation of the SARS-CoV-2 samples uniformly confirmed the results of the primary testing and sequencing for all evaluated samples. The analysis demonstrated high reliability and reproducibility of the results obtained with the rTEST COVID-19 qPCR B.1.1.7 kit.

Detection of RNase P gene showed high homogeneity in all analyzed samples, confirming the suitability of this assay as an internal control for collection and RNA extraction from a clinical sample.

Table 6.
Clinical performance of the rTEST COVID-19 qPCR B.1.1.7 kit

		rTEST COVID-19 qPCR B.1.1.7				
	Sequencing	Consensus S gene	HV 69/70 + Y144 deletion (B.1.1.7)	HV 69/70 deletion	Other SARS-CoV-2 variant	Inconclusive
HV 69/70 + Y144 deletion (B.1.1.7)	37	37	36	0	0	1
HV 69/70 deletion	16	16	0	14	1	1
Other SARS-CoV-2 variant	12	12	0	0	12	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: vigilance@multiplexdx.com

12. Symbols

	Manufacturer
LOT	Batch number
-20°C	Recommended storage temperature
Σ	Package size
CE	This product complies with health, safety, and environmental protection standards for products sold within the EEA
	Date of manufacturing
IVD	*In vitro* diagnostic medical devices
	Attention, follow the safety instructions and operating instructions that come with this product

CE IVD Registration code: P2058A

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