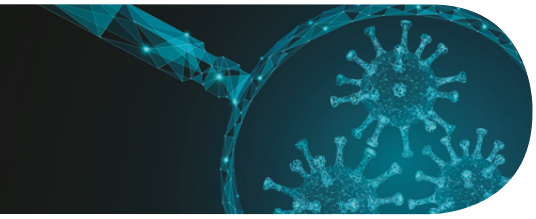

vDetect COVID-19 RT-qPCR diagnostic kit / 400 reactions

User Information

Ver. 3.0 (en), 07/2020

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
BSL: biosafety 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
DNA: deoxyribonucleic acid
DTT: Dithiothreitol
FAM: 6-carboxyfluorescein, a fluorescent reporter dye
gene E: a gene encoding the small membrane envelope protein of the SARS-CoV-2 virus
gene RdRP: a gene encoding the RNA-dependent RNA polymerase of SARS-CoV-2 virus
gene RNase P: a gene encoding human nuclear ribonuclease P
NTC: Non-Template Control
PC: Positive Control
PCR: Polymerase Chain Reaction
qPCR: Quantitative Polymerase Chain Reaction
RNA: ribonucleic acid
ROX: 6-carboxy-X-rhodamine, a fluorescent reference dye used to normalize the reporter dye signal
RT-qPCR: quantitative reverse transcription PCR
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 vDetect COVID-19 RT-qPCR diagnostic kit is a qualitative in vitro test designed to detect the presence of SARS-CoV-2 genetic material in biological samples obtained primarily from the human upper airways (nose and nasopharynx). The kit is intended exclusively for use in a diagnostic laboratory with the appropriate equipment, safety standards and properly trained personnel.

The vDetect COVID-19 RT-qPCR diagnostic kit was developed as part of a non-profit initiative with the financial support from ESET spol. s r. o.

3. Test principle

The vDetect COVID-19 RT-qPCR diagnostic kit is an improved and re-designed version of the WHO-recommended Charité, Berlin protocol. The kit contains three sets of primers and hydrolysis probes (TaqMan®) targeting the E gene, SARS-CoV-2 specific RdRP gene, and human RNase P. The full genomic RNA of SARS-CoV-2 virus spiked with human RNA is included as an internal positive control. A passive reference dye is provided as an optional reagent that may be added to normalize non-PCR related variations in fluorescence. Providing the reference dye in a separate tube enables compatibility with many real-time qPCR platforms (e.g., no ROX/low ROX/high ROX).

The RT and qPCR reactions are conducted in a one tube, 1-step fashion. The recommended workflow consists of an initial first-line screening assay for the E gene followed by a confirmatory assay for the SARS-CoV-2 specific RdRP gene. A third assay for the human RNase P gene can be used for secondary confirmation of negative samples as well as to validate sample collection, RNA extraction, and performance of the test.

One package of the kit is sufficient for 400 testing reactions. This kit offers flexibility in testing by permitting either single gene screening or precision dual gene diagnostics. E or RdRP gene (based on the end-user preference) can be exclusively used for 400 screening tests. This option is recommended when many symptomatic patients have to be screened at once. The precision dual gene option is based on running 200 screening assays first (E or RdRP gene) followed by 200 confirmation assays to confirm positive screening results (E or RdRP gene) or eliminate false-positive results. In addition, conducting an internal control assay for human RNase P assesses appropriate swab and RNA extraction, the presence of human RNA within the extracted sample, while eliminating false-negative results. Thus, for the precision dual gene diagnostics workflow, two testing reactions (screening and confirmation) should be performed per one patient and this option is recommended when precision testing is essential.

NOTICE!

- **Read all instructions in this user manual before starting work.**
- **Never use all primer sets and TaqMan® probes in one multiplexed reaction.**

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 SARS-CoV-2_E Primers/Probe Mix (must be dissolved in 0.8 ml of PCR water)
- 1x SARS-CoV-2_RdRP Primers/Probe Mix (must be dissolved in 0.8 ml of PCR water)
- 1x Human RNase P Primers/Probe Mix (must be dissolved in 0.5 ml of PCR water)
- 1x 200 µl PC SARS-CoV-2 BMC5
- 2x 2 ml 2X Brilliant III QRT-PCR Master Mix
- 1x 400 µl RT/RNase Block
- 1x 100 µl DTT (100 mM)
- 1x 100 µl Reference Dye (1mM)
- 1x 5 ml PCR water
- 1x Instructions for use

5. Storage and shelf life:

All kit components must always be transported and stored at -20 °C in the dark. After thawing, the 2X master mix may be stored at 4 °C for up to three months or returned to -20 °C for long term storage. The reference dye is light sensitive and should be kept away from light whenever possible. The shelf life of the kit is 12 months starting from the date of manufacture. The exact expiration date of the kit is indicated on the kit box/outer package. The exact expiration date of the individual components of the kit is indicated on the vials.

CAUTION!

Do not use the kit after the expiry date, which is stated on the outer carton.

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 prevents repeated thawing and freezing of the individual components, which may lead to reduced efficiency, and minimizes the need to reopen individual tubes and thus minimizes the risk of contamination of the kit components. 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. two 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 it is completely homogeneous. In the case of tubes containing enzymes (2X Brilliant III Ultra-Fast QRT-PCR Master Mix and RT/RNase Block), we recommend mixing by rotating the tube several times, pulse vortexing, or short vortexing for no longer than 5 seconds without generating any bubbles. After sufficient mixing, all tubes should be briefly spun down using a mini centrifuge. 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: vDetect COVID-19 RT-qPCR diagnostic kit has been validated and tested on Agilent devices - Mx3005P® and AriaMx®, Thermo Fisher Scientific - QuantStudio™ 5, and BioRad - CFX96 ™
- 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 without DNA, RNA, DNases and RNases: reagent tubes, PCR tubes, PCR strips and caps, PCR plates, PCR foils, sterile pipette tips with filter
- Autoclavable adjustable micropipettes
- Bio-waste container
- Autoclavable test tube racks
- PCR cooling rack

7. Workflow

CAUTION!

Work with the kit must be performed by qualified personnel.

NOTICE!

- **Workspaces must be arranged in such a way that there are separate rooms (zones) for the isolation of nucleic acids, for the preparation of amplification reactions, and for the 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.**
- **Maintain separate, dedicated laboratory equipment and consumables for each step in the workflow: nucleic acid isolation, preparation of amplification reactions, or amplification and detection of amplification products.**
- **Use separate, designated lab coats, gloves, and all other personal protective equipment for each step in the workflow: nucleic acid isolation, preparation of amplification reactions, or amplification and detection of amplification products. 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 vDetect COVID-19 RT-qPCR 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 so 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 routine vDetect COVID-19 RT-qPCR kit workflow begins with a screening test to determine the presence of viral RNA for the E or RdRP gene (order is based on user preference). Next, a confirmatory test is conducted to determine the presence of the second viral RNA gene (either RdRP or E depending on user preference). Lastly, an internal control test for human RNase P is conducted to examine swab and RNA extraction efficiency, assay performance, and as a secondary confirmation of negative results.

7.3. Workspace preparation

Before starting the protocol, clean the working space of the laminar flow box and adjacent surfaces first with a 10% solution of sodium hypochlorite (bleach) and then with a 70% solution of ethanol to remove residual bleach. Start 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 (2X Brilliant III Ultra-Fast QRT-PCR Master Mix, RT/RNase Block) 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 are supplied in lyophilized form to increase stability. It is therefore necessary to dissolve mixtures of primers and probes in PCR water when the kit is used for the first time.

Add 800 µl of the provided PCR water to each tube containing SARS-CoV-2_E and SARS-CoV-2_RdRP Primers/Probe mix (400 reactions) and 500 µl of the provided PCR water to the tube containing human RNase P Primers/Probe mix (250 reactions).

Thoroughly vortex the contents of the tubes then briefly centrifuge the tubes to remove droplets from inside 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, return kit components to the freezer (at –20 °C) or to the fridge (4 °C) (after thawing, the 2X master mix may be stored at 4 °C for up to three months or returned to –20 °C for long term storage). 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 and exclusively for one analysis at a time. Use pipettes designated exclusively for reagent preparation and use disposable filter tips that are 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 reagents from kits from different manufacturers.**
- **Do not combine or mix reagents from different lots.**

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:

Kit component	Component volume per reaction		
	E gene	RdRP gene	RNase P
PCR water**	1.5 - 1.8 µl**	1.5 - 1.8 µl**	1.5 - 1.8 µl**
2X Brilliant III Ultra-Fast QRT-PCR Master Mix	10 µl	10 µl	10 µl
SARS-CoV-2_E or SARS-CoV-2_RdRP or Human RNase P	2 µl	2 µl	2 µl
100 mM DTT	0.2 µl	0.2 µl	0.2 µl
Diluted ROX (optional)*	0.3 µl	0.3 µl	0.3 µl
RT/RNase Block	1 µl	1 µl	1 µl
Total volume	**15 µl**	**15 µl**	**15 µl**

Table of calculated volumes for a given number of reactions:

Kit components	1	2	3	4	5	6	7	8	9	10	96
2X Brilliant Ultra-Fast QRT-PCR Master Mix	10 µl	20 µl	30 µl	40 µl	50 µl	60 µl	70 µl	80 µl	90 µl	100 µl	960 µl
RT/RNAse Block	1 µl	2 µl	3 µl	4 µl	5 µl	6 µl	7 µl	8 µl	8 µl	10 µl	96 µl
100 mM DTT	0.2 µl	0.4 µl	0.6 µl	0.8 µl	1 µl	1.2 µl	1.4 µl	1.6 µl	1.8 µl	2 µl	19.2 µl
Diluted ROX (optional)*	0.3 µl	0.6 µl	0.9 µl	1.2 µl	1.5 µl	1.8 µl	2.1 µl	2.4 µl	2.7 µl	3 µl	28.8 µl
SARS-CoV-2_E or SARS-CoV-2_RdRP or Human RNase P	2 µl	4 µl	6 µl	8 µl	10 µl	12 µl	14 µl	16 µl	18 µl	20 µl	192 µl
PCR Water**	1.5 - 1.8 µl	3 - 3.6 µl	4.5 - 5.4 µl	6 - 7.2 µl	7.5 - 9µl	9 - 10.8µl	10.5 - 12.6µl	12 - 14.4 µl	13.5 - 16.2 µl	15 - 18 µl	144 - 172.8 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**

*Prepare fresh dilutions of the reference dye prior to setting up the reactions, and keep all tubes containing the reference dye protected from light exposure as much as possible. The diluted reference dye, if stored in a light-protected tube at 4 °C, can be used within the day for setting up additional assays. Make initial dilutions of the reference dye using nuclease-free PCR-grade water. If using a StepOnePlus or 7900HT Fast instrument, dilute the dye 1:50 for a final concentration of 300 nM in the reactions. For the Agilent Mx instruments (including Aria) or the ABI 7500 Fast instrument, dilute the dye 1:500 for a final concentration of 30 nM. The Bio-Rad CFX96, the Roche LightCycler® 480, and the QIAGEN Rotor-Gene Q instruments do not require the use of the reference dye.

**The volume of PCR water depends on the use of diluted ROX. If ROX is used as a reference dye, pipette 1.5 µl of PCR water per reaction, otherwise use 1.8 µl for the total mix volume of 15 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.**

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 inside 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 positions 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 SARS-CoV-2 BMC5), 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 non-template control (NTC) containing PCR water is used as a negative control instead of an unknown sample. A separate NTC for each gene (E, RdRP, and RNase P) tested must be included.
A minimum of one positive control 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 SARS-CoV-2 BMC5) is used instead of an unknown sample. A separate positive control for each gene (E, RdRP, and RNase P) tested must be included.

The PC SARS-CoV-2 BMC5 consists of isolated genomic RNA of SARS-CoV-2 spiked with human RNA. The PC SARS-CoV-2 BMC5 will yield a positive result with all primer and probe sets (i.e., 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**

Temperature conditions:
- **Reverse transcription: 50 °C, 30 min**
- **Initial denaturation: 95 °C, 3 min**
- **Cycling, 45 cycles:**
  - **Denaturation: 95 °C, 5 s**
  - **Annealing/extension: 60 °C, 20 s**

Optical channels:
- Optical channel for FAM label: blue or green channel according to the real-time PCR device used - excitation maximum around 495 nm, emission maximum around 520 nm
- Optical channel for ROX dye: orange or red channel according to the real-time PCR device used - excitation maximum around 575 nm, emission maximum around 605 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 fluorescent signal of the ROX dye does not interfere with the detection channel of the FAM fluorescent label. As a result, the vDetect COVID-19 RT-qPCR kit is fully compatible with real-time PCR devices that do not support the ROX dye normalization function.

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 and in accordance with your internal procedures for this type of assay.

- **Expected performance of controls Included in vDetect COVID-19 RT-qPCR test.**

Control Type	External Control Name	Used to Monitor	SARS-CoV-2 E	SARS-CoV-2 RdRP	Human RNase P	Expected Ct Values
Positive	PC-SARS-CoV-2 BMC5	Substantial reagent failure including primer and probe integrity	+	+	+	Ct < 35.00
Negative	NTC	Reagent and/or environmental contamination	–	–	–	None detected

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

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.

- **SARS-CoV-2 Positive Control (PC-SARS-CoV-2 BMC5)**
  The PC-SARS-CoV-2 BMC5 consists of isolated genomic RNA of SARS-CoV-2 spiked with human RNA. The PC-SARS-CoV-2 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 SARS-CoV-2 BMC5 positive control should exhibit a Ct lower than 35.00 for all E, RdRP and RNase P genes. The signal level (i.e., the relative fluorescence without normalization with the ROX dye) 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 (Extraction Control)**

  » All clinical samples should exhibit fluorescence amplification curves in the RNase P reaction that cross the threshold line within 40.00 cycles (Ct < 40.00), thus indicating the presence of the human RNase P gene RNA in the 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 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. If residual specimen is available, repeat the RNA extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

- **SARS-CoV-2 markers (E and RdRP)**

  » When all controls exhibit the expected performance, a specimen is considered negative if both SARS-CoV-2 gene (E, RdRP) amplification curves 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 40.00 cycles (Ct < 40.00).

  » When all controls exhibit the expected performance, a specimen is considered positive if both SARS-CoV-2 gene (E, RdRP) amplification curves 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 the SARS-CoV-2 genes (E, RdRP) AND the RNase P marker DO NOT cross the cycle threshold line within 40.00 cycles (Ct < 40.00), 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 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 genes (E or RdRP but not both) crosses the threshold line within 40.00 cycles (Ct < 40.00) the result is considered inconclusive. The extracted RNA should be retested. 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.

- **vDetect COVID-19 RT-qPCR diagnostic test results interpretation guide**
  The table below lists the expected results

for the vDetect COVID-19 RT-qPCR test diagnostic panel performed in dual precision mode. 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.

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 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 further guidance.
-	+	+/-			
-	-	+	SARS-CoV-2 not detected	Not detected	Report results to sender. Consider testing for other respiratory viruses.[b]
-	-	-	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.

[b] Optimal 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 disease 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 vDetect COVID-19 RT-qPCR diagnostic kit is designed for qualified and trained laboratory personnel with sufficient experience in real-time RT-qPCR testing techniques.**

9. Functional characteristics

9.1. Limit of Detection

Evaluation of analytical sensitivity (detection limit) was performed on the screening test for the E gene as well as the confirmatory test for the RdRP gene. The test was performed using the positive control „SARS-CoV-2 Standard" (Exact Diagnostics, http://www.exactdiagnostics.com/ sars-cov-2-standard.html), which in the undiluted state contains 200 copies of synthetic template per 1 µl. Dilutions were prepared by serial dilutions of the stock standard, resulting insamples with concentrations of 8 copies/µl (= 40 copies/reaction), 2 copies/µl (= 10 copies/reaction), 0.8 copies/µl (= 4 copies/reaction) and 0.4 copies/µl (= 2 copies/reaction) that were used in the analytical sensitivity test. A dilution medium was prepared by spiking commercial Human genomic DNA (Promega G3041, diluted to 500 ng/ml) into sodium citrate buffer (pH 6.4) and was used to dilute the control material.
The dilution medium also served to mimic as closely as possible the characteristics of the standard clinical samples as a complex mixture, including the possible content of RT-qPCR inhibitors. The assay was performed in eight replicates for each prepared dilution.

The test confirmed the high sensitivity of the vDetect COVID-19 RT-qPCR diagnostic kit. Reliable template detection, of both E and RdRP genes was demonstrated down to 2 copies per reaction (0.4 copies/µl).

9.2. Test specificity

Evaluation of specificity (cross-reactivity to other coronaviruses) was performed on the screening test for the E gene as well as the confirmatory test for the RdRP gene. 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, MERS-CoV and SARS-CoV-2, each in a separate tube. The assay was performed in three replicates for each of the indicated viruses.

The test confirmed the high specificity of the vDetect COVID-19 RT-qPCR diagnostic kit. A positive result was recorded exclusively in reactions containing SARS-CoV-2 RNA 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 disease, 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 vDetect COVID-19 RT-qPCR kit was designed not to amplify the most commonly used synthetic positive controls at all or only with low efficiency. Thus, the vDetect COVID-19 RT-qPCR 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 (non-specific interaction between oligonucleotides contained in the individual primer and probe mixtures) was performed on the screening test for the E gene as well as the confirmatory test for the RdRP gene. The test was performed as an analysis of multiple non-template controls (NTC), including 20 replicates for the E gene and 40 replicates for the RdRP gene.

	E gene			RdRP gene			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	Total number of replicates	Number of reactions with positive results	Detection success
40 copies/reaction	8	8	100%	8	8	100%	8	8	100%
10 copies/reaction	8	8	100%	8	8	100%	8	8	100%
4 copies/reaction	8	8	100%	8	8	100%	8	8	100%
2 copies/reaction	8	8	100%	8	8	100%	8	8	100%

The assay confirmed the high chemical stability of the oligonucleotides contained in the vDetect COVID-19 RT-qPCR 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

Clinical evaluation of the performance of the vDetect COVID-19 RT-qPCR kit was performed on the screening test for the E gene as well as the confirmatory test for the RdRP gene. The evaluation was performed on a selected set of 38 positive and 54 negative clinical samples of patients, whose COVID-19 status was confirmed by an RT-qPCR reference method used for routine testing by the regional public health authorities of the Slovak Republic. Testing of this selected set of samples was performed independently in two laboratories, with one of the laboratories working with blinded samples.
Independent analyses in both laboratories clearly confirmed the results of the reference method for all samples evaluated and demonstrated the high reliability and reproducibility of the results obtained with the vDetect COVID-19 RT-qPCR kit (see table below). Based on the data obtained, the vDetect COVID-19 RT-qPCR kit has 100% diagnostic sensitivity and 100% diagnostic specificity.

	Reference method	vDetect COVID-19 RT-qPCR kit laboratory 1		vDetect COVID-19 RT-qPCR kit laboratory 2	
		E gene	RdRP gene	E gene	RdRP gene
Number of correctly identified positive samples	38	38	38	38	38
Number of false positive samples	-	0	0	0	0
Number of correctly identified negative samples	52	54	54	54	54
Number of false negative samples	-	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 2 205 35 649
Email: vdetect@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: P1048A

MultiplexDX, s. r. o.
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