COVID-19 *direct* RT-PCR+

For use on a qPCR device

In-vitro-Diagnostic IVD

Instructions for Use

Purpose

COVID-19 *direct* RT-PCR+ is an *in vitro* real-time test based on the reverse transcription polymerase chain reaction (RT-PCR) for the qualitative detection of nucleic acid from SARS-CoV-2 and **SARS-CoV-2-variant N501Y** in samples of the upper and lower respiratory tract (e.g. nasopharyngeal or oropharyngeal swabs, lower respiratory aspirates, bronchoalveolar lavage, mouth/throat rinse solution and nasal lavage) from individuals suspected of having COVID-19 collected by their healthcare provider.

The results are used to identify SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of the infection. Positive results indicate the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine the patient's infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The pathogen detected may not be the final cause of the disease.

Negative results do not rule out infection with SARS-CoV-2 and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history and epidemiological information.

The COVID-19 *direct* RT-PCR+ test is intended for use by laboratory personnel specifically trained in RT-PCR and *in vitro* diagnostic techniques.

Summary and testing principle

Field of application

Coronavirus disease (SARS coronaviruses such as SARS-CoV-2, pathogen of the COVID-19 pandemic) is an infectious disease caused by a newly discovered coronavirus. The virus is mainly transmitted by droplet infection when an infected person coughs, sneezes or exhales. Symptoms can range from fever, cough and breathing difficulties to pneumonia and acute respiratory distress syndrome, and can lead to death in comorbid persons [1].

Since mid-December 2020 there have been reports from the United Kingdom (UK) on the increasing identification and spread of the so-called SARS-CoV-2 VOC 202012/01 (VOC: variant of concern) (Public Health England, 2020; Rambaut et al., 2020). These viruses belong to the line **B.1.1.7 (501Y.V1)** and have been spreading since September 2020, mainly in the south and south-east of Great Britain.

The virus variant **B.1.351 (501Y.V2)** from South Africa (SA) was also first reported in December 2020. Initial data indicate that this variant can be viewed as more transferable. Both variants are characterised by an unusually high number of non-synonymous polymorphisms in the spike protein.

N501Y: Increasing the affinity for the cellular ACE2 receptor protein.

Testing principle explanation

The COVID-19 *direct* RT-PCR+ test is designed to detect SARS-CoV-2 and SARS-CoV-2 mutation N501Y directly from the swab or alternatively from samples generated by using mouth and throat rinsing solutions on a commercially available qPCR cycler.

It consists of a test kit with two solutions to be mixed before use and filled into the PCR reaction tubes or wells of the microtiter plates. The ready-to-use solutions are available in portions for 96 tests each. They contain all necessary chemicals (buffer components, substances for reverse transcription and PCR incl. real-time probes with fluorescent labelling). After adding the untreated patient sample to the PCR reaction tubes, the analysis is performed directly on the qPCR device.

The COVID-19 *direct* RT-PCR+ test contains detection Probes specific for SARS-CoV-2 and Probes for the detection of the subgenus Sarbecovirus, the mutation N501Y as well as an Internal Control. The Probes are each labelled with fluorescent reporter dyes (FAM for SARS-COV-2, N-gene; CalFluor Red 610 for Sarbecovirus, E-gene; **Atto647N for SARS-CoV-2 mutation N501Y, Spike-gene;** HEX for Internal Control). Each Probe also has a second dye which acts as a quencher and suppresses the fluorescent signals of the intact Probes.

During PCR amplification, the Probes hybridise to a specific target sequence, if present in the sample. This leads to the cleavage of the Probe by the 5'-to-3'-exonuclease activity of the DNA polymerase and thus to the separation of the reporter and quencher dyes, which in turn leads to an increase in the fluorescence signal. With each PCR cycle, more and more cleaved Probes are produced and the signal continues to increase. Each reporter dye is measured at a defined wavelength, which allows simultaneous detection and differentiation of different target sequences in the corona virus as well as Internal Control. The analysis is performed semi-quantitatively by comparing Ct (cycle threshold) values. The Ct value describes the cycle in which the signal rises above a certain threshold for the first time. The more target copies (here: virus RNA) are present in the sample, the lower the value.

To ensure that the patient sample does not contain any RT-PCR-inhibiting substances, an Internal Control is added to each reaction. The Internal Control is an artificial RNA target, which has no known homologies and is contained in the same RT-PCR reaction mixture. It is transcribed into cDNA, amplified and detected. In this way, false negative test results due to RT-PCR inhibition can be excluded.

The buffer contains additives for virus lysis, enhancement of PCR efficiency and inhibition of RNases. Patient samples can be analysed directly without prior RNA extraction.

Reagents, material

Package contents

The reagents of each vial set (solution A+, solution B+) are sufficient for 96 determinations. Each reagent set contains:

Material	REF	Quantity
Solution A+	DNA-Polymerase, Reverse Transcriptase, Probes for E-gene, Probes for N-gene, Probes for SARS-CoV-2 mutation N501Y, dNTPs, Additives, Reaction Buffer (item# FBC102)	1 x 1.6 mL/96-Kit
Solution B+	Probes for Internal Control, Additives for the reduction of Inhibition, Reaction Buffer (item# FBC102)	1 x 18 µL/96-Kit
Negative Control	Item# FBC101-NC	1 (optional), 13 µL/96-Kit)
Positive Control	Item# FBC101-PC	1 (optional), 13 µL/96-Kit)
Quick Start Protocol	--	1 (instruction manual)

Additional material, equipment and software required:

- qPCR cycler
- disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus adhesive optical film
- Pipettes
- Pipette tips
- Cooling unit
- Vortex mixer, optional

Shelf life and handling

Reagent	Storage temperature	Handling
Solution A+	-25 °C to -18 °C	+2 °C to +8 °C
Solution B+	-25 °C to -18 °C	+2 °C to +8 °C

Repeated thawing and freezing of the components - more than once - must be avoided. If necessary, aliquoting of the test components after the first thawing is recommended. The package bears an expiry date, after which no quality guarantee can be given.

The test should only be performed by trained and authorised personnel. Cross-contamination can lead to false test results. Add patient samples and controls carefully. Ensure that reaction preparations are not carried over from one well to the next. If the user makes substantial changes to the product or the application instructions, results may not correlate with the intended use.

Warnings and safety precautions

The COVID-19 *direct* RT-PCR+ test is intended for *in vitro* diagnostic use only.

- All patient samples must be treated as potentially infectious material.
- Suitable disposable protective gloves must be worn throughout the test procedure.
- All reagents and materials that come into contact with potentially infectious samples must be treated with appropriate disinfectants or disposed of according to hygiene regulations. The concentration specifications and incubation times of the manufacturers must be followed.
- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to incorrect results.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging film.
- Protect reagents from heat and moisture.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Good laboratory practice is essential for optimum test performance. Due to the high analytical sensitivity of the test, care must be taken to ensure the purity of materials and reagents.
- If other PCR tests are performed at a generally accessible laboratory area, care must be taken to ensure that the test is not contaminated. Contamination of the test by microorganisms and desoxyribonucleases (DNases and RNases) must always be avoided.
- The laboratory should carry out routine environmental checks to minimise the risk of cross-contamination.
- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- Performing the COVID-19 *direct* RT-PCR test beyond the recommended time frame and temperature range for sample transport and storage may result in invalid results. Tests that are not completed within the specified time frames should be repeated.
- Good laboratory practice should be followed during the test.
- Dispose of unused tests according to local, regional or national regulations.
- The test kits are intended for single use and must not be reused.
- If contamination of the qPCR device is suspected, cleaning and maintenance must be carried out according to the qPCR system manual.
- Safety Data Sheets (SDS) are available on request from FRIZ Biochem.

To ensure that potential **RT-PCR-inhibiting substances** in the patient sample do not affect the analysis, an Internal Control is added to each reaction mixture. The Internal Control is an artificial RNA target, which has no known homologies. It is transcribed into cDNA, amplified and detected. In this way, false negative test results due to RT-PCR inhibition can be excluded.

The **positive control** (SARS-CoV-2 RNA, laboratory standard or optionally from FRIZ Biochem) must be treated as a patient sample and included in each RT-PCR run.

The **negative control** (laboratory standard or optionally from FRIZ Biochem) must be treated as a patient sample and included in each RT-PCR run.

A **failed positive or negative control** will invalidate the RT-PCR run and the results must not be reported.

Performance of the COVID-19 *direct* RT-PCR+ test

Sample collection and reagent preparation

The starting material for the COVID-19 *direct* RT-PCR+ test is 10 µL of a solubilized patient sample per reaction. The patient sample can be obtained from sputum, smear or mouth and throat rinsing solutions. Heat inactivation of the patient sample beforehand does not affect the performance of the reagents used in the test. A possible influence of the heat inactivation on the sample can not be excluded.

	Procedure
0	Thaw all reagents completely and keep them cool (+2 °C to +8 °C) before starting the test. One Positive and one Negative Control should be included in each qPCR run.
1	Prepare the reaction solution: add 15 µL of solution B+ to the vial of solution A+; mix/shake briefly and centrifuge if necessary. Do not vortex!
2	Pipette 15 µL of the reaction solution into each of the PCR reaction tubes/wells of the microtiter plate.
3	Add 10 µL of a solubilised patient sample or Positive Control or Negative Control per well.
4	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
5	Briefly centrifuge the microtiter plates or reaction vessels, if necessary.
6	Place the filled plate/reaction tubes in the qPCR cycler.

Settings of the qPCR/detection channels

The COVID-19 *direct* RT-PCR+ test was evaluated/validated with the Roche LightCycler® systems (96/480 II) and with the BioRad Opus 96™ qPCR cycler. For basic information on programming the various real-time cyclers, please refer to the user manual of the qPCR cycler used.

Reverse transcription	55 °C	10 min
Denaturation	95 °C	2 min
Amplification	45 cycles	
Denaturation	95 °C	5 sec
Amplification/Elongation	61 °C	15 sec

	SARS-general (E-gene)	SARS-CoV-2 (N-gene)	SARS-CoV-2 mutation N501Y (Spike-gene)	Internal Control
Reporter dye	**CalFluor Red 610**-probe	**FAM**-probe	**Atto647N**-probe	**HEX**-probe
Colour	red	green	red	yellow
Emission	610 nm	510 nm	664 nm	580 nm
Quencher	Black Hole Quencher	Black Hole Quencher	Black Hole Quencher	Black Hole Quencher

The information on detection channel wavelengths refers to the LightCycler® 96 from Roche. For the LightCycler® LC480 I and LC480 II a colour compensation is needed. If you have any questions, please contact Service at FRIZ Biochem.

Results

Validation of the results

- The negative control must be below threshold.
- The Internal Control (IC) in the negative control must show a positive curve. If the negative control shows a positive curve (contamination) or the IC in the negative control is not valid, the test run cannot be evaluated.

- The positive control must show a positive curve. The Ct value of the positive control must correspond to the laboratory standard (optional, when using FRIZ Biochem Positive Control the Ct value must be < 33). A positive control with a higher Ct value indicates amplification problems.
- The IC for negative samples must show a positive curve.
- The signal from the IC in a patient sample must be compared to the signal from the IC in the negative control. If the Ct value of the IC of a sample is more than 5 times higher than the Ct value of the IC in the negative control or if the IC signal of a sample is missing, this indicates inhibition of the RT-PCR reaction. In these cases a negative test result is not valid.

Test result interpretation

Signals that are greater than the threshold are evaluated as positive results.

Interpretation of the results of the COVID-19 *direct* RT-PCR+ test:

SARS-general (E-gene, Red 610)	SARS-CoV-2 (N-gene, FAM)	Internal control (IC, HEX)	Result	Interpretation
Positive	Positive	Positive/ Negative	**Positive!**	SARS corona viruses detected in the examined material; confirmation for SARS-CoV-2. If the signal in the Atto647N channel is positive, the N501Y mutation of SARS-CoV-2 is present.
Positive	Negative	Positive/ Negative	Potentially **positive!**	SARS corona virus detected in the examined material; no confirmation for SARS-CoV-2; re-analysis of sample if necessary. If the signal in the Atto647N channel is positive, the N501Y mutation of SARS-CoV-2 is present.
Negative	Negative	Positive	**Negative!**	No SARS corona virus and no SARS-CoV-2 were found in the examined material.
Negative	Positive	Positive/ Negative	Potentially **positive!**	No SARS corona virus detected in the material examined, but SARS-CoV-2; re-analysis of sample if necessary. If the signal in the Atto647N channel is positive, the N501Y mutation of SARS-CoV-2 is present.
Negative	Negative	Negative	**Invalid!**	The test result cannot be evaluated.

The results are used to identify SARS-CoV-2 RNA and SARS-CoV-2 variant N501Y. SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of the infection. Positive results indicate the presence of SARS-CoV-2 RNA.

A negative result does not exclude the presence of SARS-CoV-2 RNA, as results depend on correct sampling, the absence of inhibitors and a sufficient amount of RNA to be detected.

Invalid results may be obtained if the sample contains inhibitors that prevent lysis, transcription and/or amplification and detection of the target nucleic acids. For information on known interfering substances, please refer to the section "procedural restrictions".

Procedural restrictions

Test results should always be seen in the context of the clinical findings. Therapeutic consequences of the diagnostic results must be drawn in relation to the clinical findings.

A negative SARS/SARS-CoV-2 test result cannot exclude an infection with the respective pathogen.

Reliable results depend on the correct procedures for sample collection, storage and handling.

Reliable results can only be guaranteed if proper procedures are used for sampling, transportation, storage and handling of the samples. Follow the procedures in these instructions for use.

This test is intended for the detection of SARS-CoV-2 RNA and SARS-CoV-2 variant N501Y in nasopharyngeal and oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, mouth/throat rinse solution and nasal irrigation/aspirate or nasal aspirator.

The test was validated with samples collected in a Copan UTM-RT System (UTM-RT), BD™ Universal Viral Transport System (UVT), in Yocon VTM/UTM, in NaCl solution, in Amies solution or in IsoFlow Sheath Fluid (Beckman Coulter). Other sample solubilisation types may lead to inaccurate results.

The detection of SARS-CoV-2 RNA and SARS-CoV-2 variant N501Y depends on the sampling method, patient factors (e.g. presence of symptoms), and/or stage of infection.

Due to the inherent differences between technologies, it is recommended that you qualify the technology before moving from one technology to the next. Users should follow their own specific guidelines / procedures.

Good laboratory practice and careful adherence to the procedures outlined in these directions for use are necessary to avoid contamination of the reagents.

The detection of SARS-CoV-2 RNA and SARS-CoV-2 variant N501Y depends on the number of organisms in the sample and can be influenced by the sampling procedure and patient-related factors.

Various interfering substances can lead to false negative or invalid results. The COVID-19 *direct* RT-PCR**+** test includes an Internal Control to detect samples containing substances that interfere with PCR amplification.

Patient samples in solutions with chaotropic salts such as guanidinium thiocyanate are not allowed.

Mutations or polymorphisms in primer and probe binding regions can interfere with the detection of new variants that may result in false negatives for the COVID-19 *direct* RT-PCR**+** test.

Analyte targets (viral sequences) may persist *in vivo* regardless of the viability of the virus (contamination). The detection of (an) analyte target(s) does not mean that a corresponding virus(s) is/are infectious, nor that they are the causative agents of clinical symptoms.

There is a risk of false positive results due to cross-contamination by target organisms, their nucleic acids or their amplified product.

This product should only be used by personnel trained in RT-PCR techniques.

For use with this product, LightCycler® LC480 II, Roche LightCycler® 96 and the BioRad CFX96™ have been validated. A different qPCR system may lead to altered Ct values and results.

Analytical Performance

The analytical performance evaluation was initially carried out using the COVID-19 *direct* RT-PCR test and describes the sensitivity and specificity of the assay with regard to SARS-CoV-2 detection excluding the mutation variant N501Y.

Comparative studies between the COVID-19 *direct* RT-PCR test and the COVID-19 *direct* RT-PCR+ show that the introduction of additional primers / probes for the detection of the SARS-CoV-2 mutation N501Y (spike gene) does not have a significant effect on the analytical sensitivity and specificity of the SARS-CoV-2 detection (E-gene and N-gene).

Analytical sensitivity:

The limit of detection (LoD) was determined with dilution series of SARS-CoV-2 RNA spiked in negative synthetic matrix with human genomic DNA (both from Exact Diagnostics) on a LightCycler® 96 System (Roche):

RNA copies/reaction	N-gene (# positive/total)	N-gene Hit rate (%)	E-gene (# positive/total)	E-gene Hit rate (%)
1	7/21	33,33	7/21	33,33
3	17/21	80,95	19/21	90,48
5	20/21	95,24	20/21	95,24
10	11/11	100,00	11/11	100,00
100	3/3	100,00	3/3	100,00
1000	3/3	100,00	3/3	100,00

The 95 % detection limit (95 CI) was determined using Logistic Regression (Logit) with GraphPad Prism 8.4.3 software.

Analytical sensitivity (LoD $_{95\ CI}$):	**N-Gen: $LoD_{95\ CI}$ = 5,85 (4,74 – 7,05) copies/reaction** **E-Gen: $LoD_{95\ CI}$ = 4,13 (3,60 – 4,67) copies/reaction**

The analytical performance of COVID-19 *direct* RT-PCR+ test using mouth/throat rinse solution (MRS) was determined with dilution series of spiked in SARS-CoV-2-variant N501Y RNA in comparison to SARS-CoV-2-variant N501Y RNA dilution series in 0,9% sodium chloride solution (NaCl; Sigma Aldrich). COVID-19 *direct* RT-PCR+ identifies SARS-CoV-2 RNA and SARS-CoV-2-variant N501Y up to a dilution of $1:10^{-6}$.

	Dilution factor	N-Gen (Ct-value)	E-Gen (Ct-value)	N501Y (Ct-value)
MRS/SARS-CoV-2	1:1.000	23,5	23,3	21,79
NaCl/SARS-CoV-2	1:1.000	22,97	22,8	21,64
MRS/SARS-CoV-2	1:10.000	26,54	26,34	25,01
NaCl/SARS-CoV-2	1:10.000	26,49	26,4	25,35
MRS/SARS-CoV-2	1:100.000	30,43	30,76	29,71
NaCl/SARS-CoV-2	1:100.000	29,97	29,9	29,31
MRS/SARS-CoV-2	1:1.000.000	34,5	40,58	37,14
NaCl/SARS-CoV-2	1:1.000.000	33,48	34,45	35,09
MRS/SARS-CoV-2	1:10.000.000	n.a.	n.a.	n.a.
NaCl/SARS-CoV-2	1:10.000.000	41,09	43,91	n.a.

Analytical specificity

In silico analyses, *in vitro* analyses of relevant human pathogenic bacteria and viruses as well as investigations of interfering substances in nasal sprays, mouth wash solutions or eye drops (all shown below) were used to access analytical specificity:

Analytical specificity:	**100 %**

The *in silico* analyses for potential cross-reactivities was performed with the organisms listed in the table below using BLAST Alignment with the sequences stored in the NCBI database. Identities of the individual primers of less than 80 % each to the target sequence were not considered significant.

In silico analyses for cross reactivity:

Organism	N-gene identity [%]		E-gene identity [%]		IC identity [%]	
	Forward Primer	Reverse Primer	Forward Primer	Reverse Primer	Forward Primer	Reverse Primer
Human coronavirus 229E	no alignment*		no alignment*		no alignment*	
Human coronavirus OC43	no alignment*		no alignment*		no alignment*	
Human coronavirus HKU1	no alignment*		no alignment*		no alignment*	
Human coronavirus NL63	no alignment*		no alignment*		no alignment*	
SARS-CoV	38,10	85,71	100,00	100,00	no alignment*	
MERS-CoV	no alignment*		no alignment*		no alignment*	
Adenovirus	no alignment*		no alignment*		no alignment*	
Humanes metapneumovirus	no alignment*		no alignment*		no alignment*	
HPIV1	no alignment*		no alignment*		no alignment*	
HPIV2	no alignment*		no alignment*		no alignment*	
HPIV3	no alignment*		no alignment*		no alignment*	
HPIV4	no alignment*		no alignment*		no alignment*	
Influenza A Virus	no alignment*		57,69	81,82	no alignment*	
Influenza B Virus	no alignment*		no alignment*		no alignment*	
Influenza C Virus	no alignment*		no alignment*		no alignment*	
Enterovirus/Rhinovirus	no alignment*		no alignment*		no alignment*	
Respiratory syncytial virus	no alignment*		no alignment*		no alignment*	
Rubella Virus	no alignment*		no alignment*		no alignment*	
Parechovirus	no alignment*		no alignment*		no alignment*	
Chlamydia pneumoniae	no alignment*		no alignment*		54,55	85,71
Haemophilus influenzae	no alignment*		no alignment*		no alignment*	
Legionella pneumophila	no alignment*		no alignment*		no alignment*	
Mycobacterium bovis subsp. Bovis	no alignment*		no alignment*		no alignment*	
Streptococcus pneumoniae	no alignment*		no alignment*		no alignment*	
Streptococcus pyogenes	no alignment*		no alignment*		no alignment*	
Bordetella pertussis	no alignment*		no alignment*		no alignment*	
Mycoplasma pneumoniae	no alignment*		no alignment*		no alignment*	
Pneumocystis jirovecii	no alignment*		no alignment*		no alignment*	
Candida albicans	no alignment*		no alignment*		no alignment*	
Corynebacterium diphtheriae	no alignment*		no alignment*		no alignment*	
Bacillus anthracis	no alignment*		no alignment*		no alignment*	
Moraxella catarrhalis	no alignment*		no alignment*		no alignment*	

Organism	N-gene identity [%]		E-gene identity [%]		IC identity [%]	
	Forward Primer	Reverse Primer	Forward Primer	Reverse Primer	Forward Primer	Reverse Primer
Neisseria elongata	no alignment*		no alignment*		no alignment*	
Neisseria meningitidis	80,95	57,14	no alignment*		no alignment*	
Pseudomonas aeruginosa	no alignment*		no alignment*		no alignment*	
Staphylococcus aureus	no alignment*		no alignment*		no alignment*	
Staphylococcus epidermidis	no alignment*		no alignment*		no alignment*	
Streptococcus salivarius	no alignment*		no alignment*		no alignment*	
Leptospiraceae	76,19	80,95	no alignment*		no alignment*	
Chlamydia psittaci	no alignment*		no alignment*		no alignment*	
Coxiella burnetii	no alignment*		no alignment*		no alignment*	
Legionella	no alignment*		no alignment*		no alignment*	
Leptospira	76,19	80,95	no alignment*		no alignment*	
Mycobacterium tuberculosis	no alignment*		no alignment*		no alignment*	

* Forward and reverse primers have a sequence identity to the target of less than 80% each

The *in silico* analyses showed an identity of more than 80 % in only a few organisms: For the N-gene the reverse primer showed a sequence identity of more than 80 % to SARS-CoV, but the identity of the forward primer is only 38 % and the last bases at the 3'-end of both primers are not conserved. For the E-gene, the reverse primer showed a sequence identity of over 80 % to influenza A, but both the forward primer and the probe have a sequence identity of only 58 %. All other sequence identities of more than 80 % in the analysis showed at no amplification is possible due to the mapped distance of the primers to each other.

In silico analyses showed that the used primers and probes of the COVID-19 *direct* RT-PCR test specifically detect SARS-CoV-2. In addition, the specificity of the test was determined *in vitro* by examining human pathogenic bacteria and viruses. The measurement was performed in triplicates. Heat-inactivated SARS-CoV-2 viral material was used as a positive control. The following table gives an overview of the microorganisms tested for cross-reactivity:

Organism	Quantity per PCR	Result N-gene	Result E-gene
Acinetobacter baumannii (gDNA)	1.0E+06 Genome copies	Negative	Negative
Bacillus cereus (gDNA)	1.0E+06 Genome copies	Negative	Negative
Corynebacterium bovis (gDNA)	1.0E+06 Genome copies	Negative	Negative
Enterococcus faecium (gDNA)	1.0E+06 Genome copies	Negative	Negative
Enterovirus D68 (gRNA)	1.0E+05 Genome copies	Negative	Negative
Haemophilus influenzae (gDNA)	1.0E+06 Genome copies	Negative	Negative
Human coronavirus, NL63 (gRNA)	1.0E+05 Genome copies	Negative	Negative
Human coronavirus, 229E (gRNA)	1.0E+05 Genome copies	Negative	Negative
Human respiratory syncytial virus (gRNA)	1.0E+05 Genome copies	Negative	Negative
Influenza A RNA H3N1 Panel (gRNA)	19,9 Ct	Negative	Negative
Influenza A Virus, H3N2 (gRNA)	1.0E+05 Genome copies	Negative	Negative
Influenza A Virus, H1N1 (gRNA)	1.0E+05 Genome copies	Negative	Negative
Influenza B Virus (gRNA)	1.0E+05 Genome copies	Negative	Negative
Lactobacillus salivarius (gDNA)	1.0E+06 Genome copies	Negative	Negative

Organism	Quantity per PCR	Result N-gene	Result E-gene
Mycobacterium tuberculosis (gDNA)	1.0E+06 Genome copies	Negative	Negative
Neisseria meningitidis (gDNA)	1.0E+06 Genome copies	Negative	Negative
Pseudomonas aeruginosa (gDNA)	1.0E+06 Genome copies	Negative	Negative
Staphylococcus aureus SA113 (Culture)	1.0E+06 CFU	Negative	Negative
Staphylococcus.epidermidis M0881 (Culture)	1.0E+06 CFU	Negative	Negative
Staphylococcus.epidermidis W23144 (Culture)	1.0E+06 CFU	Negative	Negative
Streptococcus mutans (gDNA)	1.0E+06 Genome copies	Negative	Negative

None of the pathogens tested gave a positive signal in COVID-19 *direct* RT-PCR (all Internal Controls valid).

Interfering substances:
To investigate the effect of potentially interfering substances on the performance of COVID-19 *direct* RT-PCR testing, the highest concentration of the substance potentially present in nasopharyngeal samples was added to a negative clinical nasopharyngeal matrix with and without heat-inactivated SARS-CoV-2 virus.

In addition, a control sample with matrix and heat-inactivated SARS-CoV-2 virus, but without potentially interfering substance was used as reference. Each condition was tested in triplicates. The following table gives an overview of the potentially interfering substances tested with the COVID-19 *direct* RT-PCR test, their active ingredient, the amount of medication applied as well as the COVID-19 *direct* RT-PCR test results.

Potentially interfering substances:

Trade name	Active ingredient per unit	% (v/v)*	Result N-gene	Result E-gene	Result IC
ratioAllerg® Hay fever spray	50 µg Beclomethasone dipopionate	15	3/3	3/3	3/3
Otri-Allergie Hay fever spray	50 µg Fluticasone propionate	15	3/3	3/3	3/3
MometaHEXAL® Hay fever spray	50 µg Mometasone Fuorate	25	3/3	3/3	3/3
NASACORT® Nasal Spray	55 µg triamcinolone acetonide	20	3/3	3/3	3/3
Vividrin® akut Nasal Spray	140 µg Azelastine hydrochloride	20	3/3	3/3	3/3
Nasivin® Nasal Spray	22,5 µg Oxymetazoline hydrochloride	10	3/3	3/3	3/3
NASENSPRAY Heumann	90 µg Xylometazolinhydrochlorid	15	3/3	3/3	3/3
Syntaris® Nasal Spray	25 µg Flunisolide	15	3/3	3/3	3/3
Rhinocort Topinasal Nasal Spray	64 µg Budesonide	10	3/3	3/3	3/3
CromoHEXAL® Eye drops	ca 400 µg Sodium agglomoglicate	5	3/3	3/3	3/3
Tobrex® Eye drops	ca 60 µg Tobramycin	5	3/3	3/3	3/3
LISTERINE® Cool Mint Mouthwash	Ethanol (N.N.)	50	0/3	0/3	0/3
		25	3/3	3/3	3/3

Trade name	Active ingredient per unit	% (v/v)*	Result N-gene	Result E-gene	Result IC
Chlorhexamed® FLUID 0,1 %	15 µg Chlorhexidine bis(D-gluconate)	50	3/3	3/3	3/3
Dequonal® Solution	1,5 mg Dequalinium chloride , 3,5 mg Benzalkonium chloride	50	3/3	3/3	3/3
Octenident® Mouth rinse solution	Octenidine (N.N.)	50	3/3	2/3	0/3
		25	3/3	3/3	3/3

* concentration of medication in the medication-matrix mixture

With the exception of two mouth rinses (LISTERINE® Cool Mint and Octenident®) at their highest tested concentration, SARS-CoV-2 positive and negative samples (the latter not shown) were clearly identified in the presence of the tested potentially interfering substances. In the case of LISTERINE® Cool Mint and Octenident®, additional concentrations were therefore tested. For both mouth rinses, N-gene, E-gene and internal control were detectable from a concentration of 25 % (v/v).

Clinical performance

The clinical performance evaluation of COVID-19 *direct* RT-PCR+ test was carried out in two laboratories (Germany, Bavaria, Munich) on anonymised samples in direct comparison to standard laboratory analysis (isolation/purification of RNA from patient material and subsequent RT-PCR). It included the analysis of 86 patient samples.

		Standard laboratory analysis		
		Positive	Negative	Total
COVID-19 *direct* RT-PCR+	Positive	55	1	56
	Negative	28	2	30
	Total	83	3	86

The static evaluation (via https://www.medcalc.org/calc/diagnostic_test.php) gives the following performance data:

Clinical performance data	Value	95 % CI
Sensitivity	**96,49 %**	89,89 % bis 99,57 %
Specificity	**96,55 %**	82,24 % bis 99,91 %

Note to the user

All serious incidents relating to the device must be notified to the manufacturer and the competent authority of the Member State where the user and/or patient is established.

CE This product complies with the requirements of the European Directive 98/79/EC for in-vitro diagnostics.

Symbols:

IVD	For use in in-vitro diagnostics	REF	Item number
	Manufacturer	Σ	Content sufficient for XY provisions
	Temperature limitation		Biological risk
	Can be used until	LOT	Batch designation
	Follow instructions for use	CONT	Content

Literatur:

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