# ViroReal® Kit SARS-CoV-2 & SARS

## Manual


CE

IVD **For *in vitro* diagnostic use**

| | | | |
|---|---|---|---|
| REF | **DHUV02353** | Σ | **50** |
| REF | **DHUV02313** | Σ | **100** |
| REF | **DHUV02313x5** | Σ | **500** |

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## Explanation of symbols

| | | | |
|---|---|---|---|
| LOT | Batch code | | Use by |
| REF | Catalogue number | | Manufactured by |
| Σ | Contains sufficient for <n> tests | | Store at |
| CE | This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices | IVD | For *in vitro* diagnostic use |
| | Corrosion, GHS05 | | Exclamation mark, GHS07 |

## 1. Intended use

ViroReal® Kit SARS-CoV-2 & SARS is an *in vitro* diagnostic test, based on one-step reverse transcription real-time PCR, for the detection of the N gene of SARS-CoV-2 and SARS-CoV. The kit allows rapid and sensitive detection of RNA of SARS-CoV-2 and SARS-CoV in samples purified from the respiratory tract.

## 2. Product description

ViroReal® Kit SARS-CoV-2 & SARS detects the nucleocapsid protein gene (N gene) of SARS-CoV-2 as well as SARS-CoV and SARS-related coronavirus. Other beta coronaviruses are not detected with this kit.
The primer and probe design chosen is not identical with the WHO design. ViroReal® Kit SARS-CoV-2 & SARS is intended to cover possible future changes in the virus sequence, therefore a highly conserved region in all SARS coronavirus clusters of the N gene was chosen as target region. This approach allows universal detection of all so far known SARS-CoV strains including SARS-CoV-2 and SARS-like CoV without discriminating between strains.

A probe-specific amplification curve at 530 nm (FAM channel) indicates the amplification of virus-specific RNA.
An internal RNA positive control (RNA IPC) is detected in Cy5 channel and is used as RNA extraction as well as RT-PCR inhibition control. The target for the RNA IPC is extracted with the sample.

This test has been developed for use with the Applied Biosystems® (ABI) 7500 instrument (Thermo Fisher Scientific), LightCycler® 480 II (Roche), cobas Z 480 Analyzer (Roche) and Mx3005P® (Agilent), but is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel.

The test is based on one-step reverse transcription real-time PCR (RT-PCR). A specific RNA sequence of the pathogen genome is transcribed into cDNA and amplified in a one-step PCR. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplificates

When using PCR-platforms not tested by ingenetix, an evaluation of the multiplex-PCR is recommended. Keep in mind that some PCR-platforms first have to be calibrated with the corresponding dye before performing multiplex-PCR.

Ingenetix BactoReal®, ViroReal®, MycoReal, PanReal, ParoReal and SeptiReal Kits have been optimized to run under the same thermal cycling conditions. DNA and RNA material can be analysed in one run.

## 3. Pathogen information

Coronaviruses are positive single-stranded RNA viruses of the family *Coronaviridae*. Several different strains of coronaviruses are currently known to infect humans (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, MERS-CoV, SARS-CoV, SARS-CoV-2, NcoV and HCoV-EMC). Strains HCoV-229E, HCoV-NL63, HCoV-OC43, MERS-CoV and HCoV-HKU1 cause cold, upper respiratory infection, bronchiolitis and pneumonia in humans. SARS-CoV, a beta coronavirus, causes the Severe Acute Respiratory Syndrome (SARS).
SARS-CoV-2 is a beta coronavirus that emerged in Wuhan, China in December 2019. The virus is responsible for the disease COVID-19 (corona virus disease 2019). Fever, cough and breathing difficulties are described as the most frequent initial symptoms, later on it can lead to pneumonia. The coronavirus spreads mainly by droplet and contact transmission. In most of the cases a mild course of infection can be observed, while more severe courses are observed in about 15%-20% of the cases, with a mortality rate of up to 3%.

# 4. Contents of the Kit, Stability and Storage

## 4.1. Kit contents order no. DHUV02353 and DHUV02313

| | | DHUV02353 | DHUV02313 | |
|---|---|---|---|---|
| **Component** | **Content** | **Quantity 50 reactions** | **Quantity 100 reactions** | **Storage** |
| SARS-CoV-2 & SARS Assay Mix (green cap) | Primer and probe (FAM) for virus detection | 1 x 50 µl | 2 x 50 µl | -15°C to -25°C |
| RNA IPC-3 Assay Mix (yellow cap) | Primer and probe (Cy5) for RNA IPC detection | 1 x 50 µl | 2 x 50 µl | -15°C to -25°C |
| RNA IPC Target (orange cap) | Target for RNA IPC | 1 x 100 µl | 1 x 100 µl | -15°C to -25°C |
| SARS-CoV-2 Positive Control (red cap) | RNA Positive Control ($10^3$ copies/µl) | 1 x 120 µl | 1 x 120 µl | -15°C to -25°C |
| RNA Reaction Mix (white cap) | RNA Reaction Mix | 1 x 250 µl | 2 x 250 µl | -15°C to -25°C |
| Nuclease-free water (blue cap) | Nuclease-free water | 1 x 1000 µl | 1 x 1000 µl | -15°C to -25°C |

## 4.2. Kit contents order no. DHUV02313x5

| | | DHUV02313x5 | |
|---|---|---|---|
| **Component** | **Content** | **Quantity 500 reactions** | **Storage** |
| SARS-CoV-2 & SARS + RNA IPC-3 Assay Mix (green cap) | Primer and probe for virus detection (FAM) and RNA IPC detection (Cy5) | 5 x 100 µl | -15°C to -25°C |
| RNA IPC Target (orange cap) | Target for RNA IPC | 1 x 500 µl | -15°C to -25°C |
| SARS-CoV-2 Positive Control (red cap) | RNA Positive Control ($10^3$ copies/µl) | 1 x 120 µl | -15°C to -25°C |
| RNA Reaction Mix (white cap) | RNA Reaction Mix | 5 x 500 µl | -15°C to -25°C |
| Nuclease-free water (blue cap) | Nuclease-free water | 3 x 1000 µl | -15°C to -25°C |

The components of ViroReal® Kit SARS-CoV-2 & SARS are stable until the expiry date stated on the label. Repeated freeze/thaw cycles should be avoided. Protect kit components from light.

**RNA Reaction Mix:** The Master Mix provided with the kit has been designed for reliable, high-sensitivity one-step reverse transcription real-time PCR even in the presence of common reaction inhibitors. The Master Mix contains a thermostable MMLV Reverse Transcriptase, an RNase inhibitor, a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs, ROX™ dye (passive reference) and buffer components – additives optimized to handle RT-PCR inhibitors.

## 5. Additionally required materials and devices

- Reagents and devices for RNA-extraction
- Nuclease-free water
- Disposable powder-free gloves
- Sterile filter pipette tips
- Appropriate optical 96-well reaction plates or reaction tubes with optical closing material recommended by the manufacturer of the real-time PCR instrument
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and Cy5 channel
- Optical 96 well reaction plates or optical reaction tubes

Examples of real-time PCR instruments with required dye channels FAM (510 nm) and Cy5 (667 nm): e.g. ABI® 7500, QuantStudio™ 5 or 6 with the correct colour calibration (Thermo Fisher Scientific), Mx3005P® (Agilent), LightCycler® 480 I, II or cobas z 480 Analyzer (Roche), Rotor-Gene Q 5plex (QIAGEN), CFX96 (BioRad), MIC (Corbett) or qTOWER with module 1&5 (Analytik Jena)

## 6. Precautions and safety information

- For *in vitro* diagnostic use. The use of this kit is limited to persons instructed in the procedures of real-time PCR and *in vitro* diagnostics.
- Clean benches and devices periodically.
- Use sterile filter pipette tips.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective disposable powder-free gloves when handling kit reagents and specimens.
- Use separated working areas for specimen preparation, reagent preparation and amplification. Supplies and equipment must be dedicated to each of these separate working areas and ensure workflow in the laboratory from pre- to post-PCR.
- Be careful when handling samples and positive control to avoid cross contamination. Change gloves after handling of samples or positive control. Store positive or potentially positive material separately from reagents
- Prevent contamination of work equipment and reagents with DNA/RNA, nuclease or amplification products by good laboratory practice.
- Quality of RNA has a profound impact on the test performance. Ensure that the used RNA extraction system is compatible with reverse transcription real-time PCR technology.
- Always include a negative control per PCR-run (nuclease-free water instead of sample).
- For a valid interpretation of results, a negative control should be included during RNA-extraction (e.g. extraction of water instead of sample material), in order to exclude false-positive results due to contamination with virus RNA during extraction.
- Please note the expiry date of the kit.
- Repeated thawing and freezing of kit components should be avoided. Protect kit components from light.
  **Caution:** RNA IPC Target is stored in RNA stabilizer which contains Guanidinium thiocyanate/Triton X-100 (see MSDS, www.ingenetix.com).
- Use established laboratory practices according to your local safety regulations for discarding specimens, reagents and waste.

## 7. Limitations

- Optimal performance of this test requires appropriate specimen collection, transport and storage, as well as an appropriate RNA extraction procedure. With this kit SARS-CoV-2 detection has been validated for swabs from the respiratory tract. Test performance with other specimen types has not yet been assessed.
- A negative test result does not exclude the possibility of a SARS-CoV infection, because test results may be affected by improper specimen collection, technical error, specimen mix-up or viral quantities below the assay sensitivity. The presence of PCR inhibitors may lead to invalid results.
- For this kit highly specific primers and probes have been selected. However, false-negative or less sensitive results might be obtained due to sequence heterogeneity within the target region of not yet described clinical subtypes.
- Results should be interpreted in consideration of clinical and laboratory findings.

# 8. Preparation of samples and real-time PCR

Extract samples with an RNA extraction system compatible with reverse transcription real-time PCR technology. Make sure that at least one extraction negative control (recommended), as well as one positive control (red cap) and optional one negative control (water) are included per PCR run.

Ingenetix highly recommends performing PCR analyses in duplicates, which increases the probability of pathogen detection and facilitates interpretation of results.

Just before use, thaw the RNA Reaction Mix on ice, and invert 2 to 3 times to ensure homogenous solution. The RNA Reaction Mix does not freeze at -20°C, but gelling may occur.
Best use RNA immediately after extraction and keep it on ice. Alternatively, use RNA stored at -20°C to -80°C and avoid prolonged exposure to room temperature, thaw on ice and immediately refreeze the RNA.

## 8.1. Internal RNA Positive Control (RNA IPC)

An Internal RNA Positive Control system containing the RNA IPC assay and the RNA IPC Target excludes false-negative results due to inhibition of reverse transcription real-time PCR.

→ For control of RNA extraction and PCR inhibition, the RNA IPC Target must be added during extraction. Spike 1 µl of undiluted RNA IPC Target into the sample material after the lysis buffer was added. The RNA IPC Target must not be added directly to the sample material.

## 8.2. Positive Control

SARS-CoV-2 Positive Control is an *in vitro* synthesized RNA with a concentration of $10^3$ copies/µl. It has to be stored at -20°C.

→ As positive control use 10 µl of the Positive Control.

## 8.3. Pipetting scheme for order no. DHUV02353 and DHUV02313

| | | **Per sample** |
|---|---|---|
| **Preparation of Master Mix** (mix well) | Nuclease-free Water* | 3.0 µl |
| | RNA Reaction Mix | 5.0 µl |
| | SARS-CoV-2 & SARS Assay Mix | 1.0 µl |
| | RNA IPC-3 Assay Mix | 1.0 µl |
| | **Total volume Master Mix** | **10.0 µl** |
| **Preparation of RT-PCR** | Master Mix | 10.0 µl |
| | RNA-Sample* | 10.0 µl |
| | **Total volume** | **20.0 µl** |

*10 µl sample can be used. When using a volume other than 10 µl, the volume of $H_2O$ has to be adjusted accordingly.
**If RNA IPC Target has not already been added during extraction**: add 1 µl freshly 1:500 diluted IPC Target per reaction to the mastermix.
**Caution:** undiluted RNA IPC Target inhibits the PCR reaction.

## 8.4. Pipetting scheme for order no. DHUV02313x5

| | | **Per sample** |
|---|---|---|
| **Preparation of Master Mix** (mix well) | Nuclease-free Water* | 4.0 µl |
| | RNA Reaction Mix | 5.0 µl |
| | SARS-CoV-2 & SARS + RNA IPC-3 Assay Mix | 1.0 µl |
| | **Total volume Master Mix** | **10.0 µl** |
| **Preparation of RT-PCR** | Master Mix | 10.0 µl |
| | RNA-Sample* | 10.0 µl |
| | **Total volume** | **20.0 µl** |

*10 µl sample can be used. When using a volume other than 10 µl, the volume of $H_2O$ has to be adjusted accordingly.
**If RNA IPC Target has not already been added during extraction**: add 1 µl freshly 1:500 diluted IPC Target per reaction to the mastermix.
**Caution:** undiluted RNA IPC Target inhibits the PCR reaction.

## 8.4. Programming of the temperature profile

Further information on programming the real-time PCR instrument can be found in the respective operator's manual. Keep in mind that some PCR-platforms first have to be calibrated with the corresponding dye before performing multiplex-PCR.

**Sample Volume:** 20 µl

**Temperature Profile:**

| **Program 1** | **Program 2** | **Program 3** |
|---|---|---|
| Cycles: 1<br>Analysis: None<br>**Reverse Transcription** | Cycles: 1<br>Analysis: None<br>**Polymerase Activation** | Cycles: 45<br>Analysis: Quantification<br>Acquisition at 60° |
| 50°C<br>15 min | 95°C<br>20 sec | 95°C<br>5 sec<br>60°C<br>30 sec |

For Applied Biosystems® 7500
Ramp speed: Without "fast cycling" parameter

**Note:** This temperature profile can be used for all ViroReal®, BactoReal®, MycoReal, PanReal, ParoReal and SeptiReal kits on all PCR instruments.

**Detection channels:**

**FAM-TAMRA:** Detection of SARS-CoV-2 or SARS-CoV
**Cy5-NONE:** Detection of IPC
**Passive reference dye, if required:** ROX (e.g. for Applied Biosystems® 7500)

**For cobas z 480 Analyzer (Roche):**
**FAM:** Excitation at 465 nm, Emission at 510 nm
**Cy5:** Excitation at 610 nm, Emission at 670 nm
**Passive reference dye:** None

**For LightCycler® 480 II (Roche):**
**FAM:** Excitation at 465, Emission at 510 nm
**Cy5:** Excitation at 618, Emission at 670 nm
Detection format: 2 Color Hydrolysis Probe
**Passive reference dye:** None

# 9. Interpretation of PCR-data

For analysis of PCR results gained with ViroReal® Kit SARS-CoV-2 & SARS, select fluorescence display options FAM channel for the virus target and Cy5 channel for the RNA IPC target. Samples with positive Ct/Cp-values are considered positive. Please, check amplification curves and adjust the threshold, if necessary. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control.

For a valid interpretation, the following criteria must be fulfilled:

| | **FAM channel SARS-CoV-2 and SARS-CoV target** | **Cy5 channel RNA IPC target** | **Interpretation** |
|---|---|---|---|
| Negative control | Negative | Positive[1)] | Valid |
| Positive control | Positive | Positive[1)] | Valid |
| Extraction negative control (optional) | Negative | Positive | Valid |
| Negative sample | Negative | Positive[2)] | Valid |
| Positive sample | Positive | Positive/Negative[3)] | Valid |

[1)]Only positive if the RNA IPC Target was added at a 1:500 dilution directly to the master mix
[2)]A positive signal excludes PCR inhibition. However, IPC Ct-values should show comparable results. A shift of Ct-values can indicate a partial inhibition of PCR.
[3)]High pathogen load in the sample can lead to a reduced or absent fluorescence signal of the RNA IPC.

## 9.1. Signal in FAM channel

→ RNA of SARS-CoV-2 or SARS-CoV has been amplified. The sample has to be interpreted as positive.

## 9.2. No signal in FAM channel but signal with RNA IPC

→ No SARS-CoV-2 or SARS-CoV RNA is detectable in the sample. The sample has to be interpreted as negative.
The positive signal of the RNA IPC excludes a putative RT-PCR inhibition.

## 9.3. No signals in FAM channel and no signal with IPC

→ No interpretation can be made.
Information about possible error sources and their solution can be found in 10. Troubleshooting.

# 10. Troubleshooting

## 10.1. No virus specific signal with positive control

- Incorrect programming of the temperature profile of the real-time PCR instrument.
  → Compare the temperature profile with the protocol (see 8. Preparation of real-time PCR).
- Incorrect configuration of the PCR reaction.
  → Check your work steps (see 8. Preparation of real-time PCR) and repeat the PCR, if necessary.
- The RNA IPC Target was added undiluted directly to the mastermix. The PCR reaction is therefore inhibited.
  → Freshly dilute RNA IPC Target and repeat PCR.

## 10.2. No signal with IPC and no virus specific signal with sample

- PCR reaction was inhibited. No interpretation can be made.
  → Make sure that you use a recommended method for RNA isolation and stick closely to the manufacturer's instructions.
  → If no operating mistakes during extractions can be retraced, it is recommended to repeat the PCR with lower amounts of RNA-eluate (1/5 or 1/10 of sample volume + the adequate volume of $H_2O$).
- Incorrect PCR conditions.
  → Check the RT-PCR conditions and repeat the RT-PCR, if necessary.

## 10.3. Virus specific signal with negative control

- A contamination occurred during preparation of the RT-PCR.
  → Repeat the RT-PCR with new reagents in replicates.
  → Strictly pipette the positive control at last.
  → Make sure that workspace and instruments are decontaminated at regular intervals.

## 10.4. Virus specific signal with negative control of extraction (optional)

- A contamination occurred during extraction.
  → Repeat extraction and RT-PCR using new reagents.
  → Make sure that workspace and instruments are decontaminated at regular intervals.

# 11. Specifications and performance evaluation

ViroReal® Kit SARS-CoV-2 & SARS has been evaluated with an ABI® 7500 instrument (Thermo Fisher Scientific). For further validation data contact ingenetix.

## 11.1. Kit performance

Performance of ViroReal® Kit SARS-CoV-2 & SARS with an ABI® 7500 instrument is shown in Figure 1.

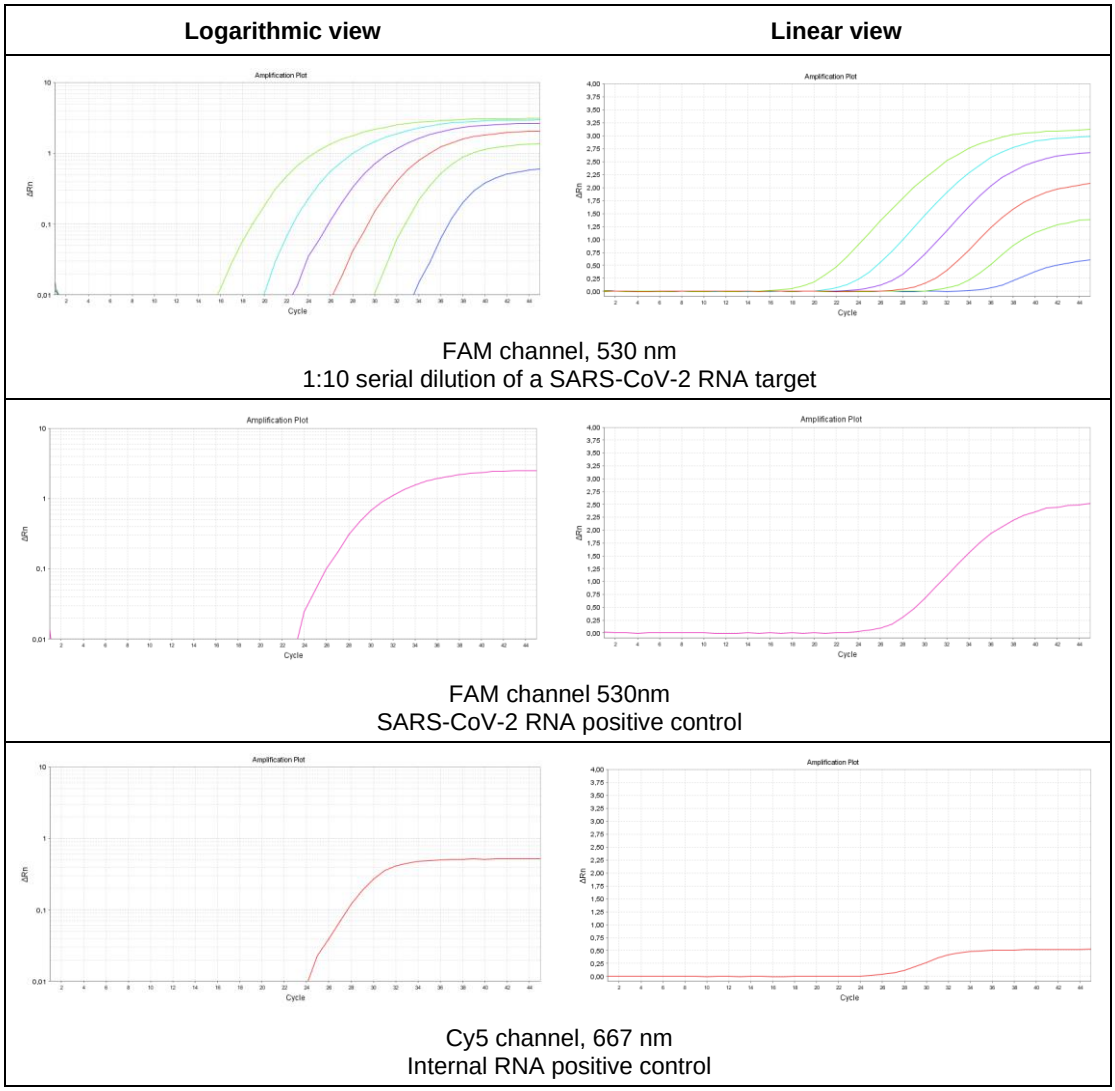


**Figure 1** Performance of ViroReal® Kit SARS-CoV-2 & SARS

## 11.2. Limit of detection and linearity

ViroReal® Kit SARS-CoV-2 & SARS was tested with a 10-fold dilution series of a synthetic RNA representing a fragment of SARS-CoV-2. At least 1 target copies/reaction could be detected.
The **limit of detection** (LoD95 = smallest number of copies of target RNA which can be detected in 95% of cases) is 13 target copies/reaction.

The assay shows **linearity** over the range of 10 to 1,000,000 target copies/ with a slope of -3.28 and a $R^2$ of 0.99 as shown in Figure 2.

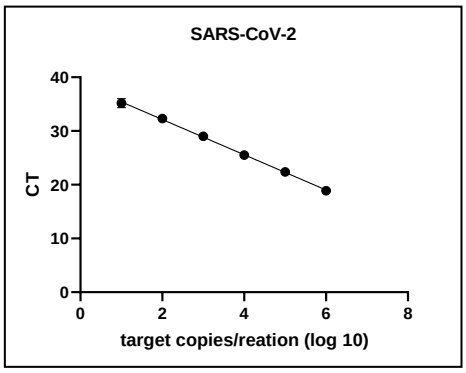


**Figure 2** Ten-fold dilution series of a SARS-CoV-2 RNA standard plotted against Ct

### 11.3. Analytical specificity

Specificity is ensured by the selection of highly specific primers and probes. A highly conserved region in all SARS coronavirus clusters of the N gene was chosen as target region. The selected primer and probes should cover possible future changes in the virus sequence and are therefore not identical with the primer and probes suggested by the WHO.
*In silico* validation of primers and probes was carried out with the basic local alignment tool (BLAST) against the NCBI database. All the data in the NCBI database showed homology in the sequence region of primer and probes throughout all SARS coronavirus clusters. This approach allows specific and universal detection of all so far known SARS coronavirus strains including SARS-CoV-2 without discriminating between strains.
ViroReal® Kit SARS-CoV-2 & SARS detects specifically the N gene of SARS-CoV-2 as well as SARS-CoV and SARS-related bat coronavirus. Other beta coronaviruses are not detected with this kit.

### 11.4. Diagnostic evaluation

ViroReal® Kit SARS-CoV-2 & SARS was tested by an external service provider (AGES, Vienna) on 94 swab samples from the respiratory tract collected from patients suspected to suffer from COVID-19. Extraction was performed with the MagNA Pure Compact using the Total Nucleic Acid Isolation Kit (Roche). Analyses were performed in single reactions on the LightCycler® 480 II instrument (Roche).
Results were compared with results of a real-time PCR test detecting the E gene of SARS-CoV-2, according to the recommendations of the WHO: Detection of 2019 novel coronavirus (2019-nCoV) (Victor M Corman et al., 2020). Fifteen out of the 94 samples were positive with both methods. One sample was positive with ViroReal® Kit SARS-CoV-2 & SARS only (Cp-value: 35.5). The remaining samples were negative with both methods.

## 12. References

Huang, Chaolin & Wang, Yeming & Li, Xingwang & Ren, Lili & Zhao, Jianping & Hu, Yi & Zhang, Li & Fan, Guohui & Xu, Jiuyang & Gu, Xiaoying & Cheng, Zhenshun & Yu, Ting & Xia, Jiaan & Wei, Yuan & Wu, Wenjuan & Xie, Xuelei & Yin, Wen & Li, Hui & Liu, Min & Cao, Bin. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 10.1016/S0140-6736(20)30183-5.

https://www.who.int/emergencies/diseases/novel-coronavirus-2019