Manual

MutaPLEX® Coronavirus

Real-Time-RT-PCR-Kit

For the simultaneous in vitro detection of RNA of novel coronavirus (SARSCoV-2) and Subgenus Sarbecovirus (SARS-CoV-1 and SARS-CoV-2), extracted from biological specimens.

Valid from 2020-04-11

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IVD

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1 INTENDED USE

The MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR kit is a screening assay for the simultaneous detection of RNA of novel coronavirus (SARS-CoV-2) and the Subgenus Sarbecovirus (SARS related Betacoronavirus: SARS-CoV-1 and SARS-CoV-2) extracted from biological specimens.

2 PATHOGEN INFORMATION

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The novel Coronavirus (SARS-CoV-2) is a new strain that has been previously identified in humans and causes the pulmonary disease CoViD-19.

Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans. Several known Coronaviruses are circulating in animals that have not yet infected humans.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Standard recommendations to prevent infection spread include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs. Avoid close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing.

3 PRINCIPLE OF THE TEST

The MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR kit contains specific primers and dual-labelled probes for the amplification of RNA (cDNA) of SARS-CoV-2 (both, RdRP gene and S gene, FAM channel) and the RNA (cDNA) of the Subgenus Sarbecoviruses (SARS-CoV-1 and SARS-CoV-2, E gene, Cy5 channel) extracted from biological specimens. Both, E gene and RdRP gene are target sequences of the viral genome recommended by the WHO. The simultaneous detection of 3 target sequences (RdRP gene, S gene and E gene) increases the diagnostic reliability, even in cases of target sequence mutations.

Furthermore, MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit contains a Control RNA (Internal Process Control, IPC), which is added during RNA extraction and detected in the same reaction by a HEX labeled probe.

The Control RNA allows the detection of RT-PCR inhibition and acts as control, that the nucleic acid was isolated from the biological specimen.

Additionally, MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit contains an Internal System Control (ISC). The ISC consists of primers and probes for the detection of a house keeping gene (Beta-actin, multi species) in the eluate from a biological specimen. The ISC helps preventing false negative results due to insufficient sample drawing or transport. The amplification of the Beta-actin target sequence is measured in the ROX channel.

4 PACKAGE CONTENTS

The reagents supplied are sufficient for 96 (KG192696), 384 (KG1926-384), or 768 (KG1926-768) reactions, respectively.

Table 1: Components of the MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR kit .

Label	Lid Colour	Content		
		96	384	768
Reaction Mix	yellow	1 x 1325 µl	4 x 1325 µl	8 x 1325 µl
Enzyme	blue	1 x 19.2 µl	1 x 76.8 µl	2 x 76.8 µl
Positive Control	red	1 x 150 µl	1 x 150 µl	2 x 150 µl
Negative Control	green	1 x 150 µl	1 x 150 µl	2 x 150 µl
Control RNA	colourless	1 x 480 µl	2 x 960 µl	4 x 960 µl

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- RNA isolation kit (e. g. MutaCLEAN® Universal RNA/DNA, KG1038)
- PCR grade water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortex
- Real time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6 TRANSPORT, STORAGE AND STABILITY

The MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR kit is shipped on dry ice or cool packs. All components must be stored at maximum -20 °C in the dark immediately after receipt. Up to 20 freeze and thaw cycles are possible. Do not use reagents after the date of expiry printed on the package.

For convenience, opened reagents can be stored at 2–8 °C for up to 6 months.

Protect kit components from direct sunlight during the complete test run.

7 WARNINGS AND PRECAUTIONS

- Stick to the protocol described in the instructions for use.
- The MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR must be performed by qualified personnel only.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organisations.

- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

8 SAMPLE MATERIAL

Starting material for MutaPLEX® Coronavirus (SARS-CoV-2) RT-PCR Kit is RNA isolated from biological specimens (e.g. swabs, sputum, stool).

9 SAMPLE PREPARATION

Commercial kits for RNA isolation such as MutaCLEAN® Universal RNA/DNA (KG1038) are recommended.

Important: In addition to the samples, always run a water control in your extraction. Treat this water control analogous to a sample.

Comparing the amplification of the control RNA in the samples to the amplification of the internal control in the water control will give insights on possible inhibitions of the Real-Time-RT-PCR. Furthermore, possible contaminations during nucleic acid extraction will be detectable.

Please note chapter 10 "Control RNA".

If the Real-Time-RT-PCR is not performed immediately, store extracted nucleic acids according to the instructions given by the extraction kit's manufacturer.

10 CONTROL RNA

A control RNA is supplied and can be used as extraction control or only as inhibition control. This allows the user to control the RNA isolation procedure and to check for possible Real-Time-RT-PCR inhibition.

a) Control RNA used as extraction control

MutaPLEX® Coronavirus (SARS-CoV-2) control RNA is added to the RNA extraction.

Add 5 µl control RNA per extraction (5 µl x (N+1)). Mix well. Perform the RNA isolation according to the manufacturer's instructions. Please follow protocol A.

The control RNA must be added to the lysis buffer of the extraction kit.

b) Control RNA used as internal control of the Real-Time-RT-PCR

If only inhibition will be checked, please follow protocol B.

11 REAL-TIME-RT-PCR

11.1 Important points before starting

- Please pay attention to chapter 7 "Warnings and precautions".
- Before setting up the Real-Time-RT-PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the RT-PCR set up.
- In every RT-PCR run, one positive control and one negative control should be included.
- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed and centrifuged very briefly.
- Due to the high viscosity of the enzyme (blue lid), prewarming at room termperature for 15 min is recommended.
- We recommend to keep reagents and samples at 2–8 °C (e. g. on ice or a cooling block) at all times.

11.2 Procedure

If the control RNA is used to control both, the Real-Time-RT-PCR and the RNA isolation procedure, please follow protocol A. If the control RNA is solely used to detect possible inhibition of the Real-Time-RT-PCR, please follow protocol B.

Protocol A

The control RNA was added during RNA extraction (see chapter 10 "Control RNA"). In this case, prepare the master mix according to table 2.

The master mix contains all of the components needed for RT-PCR except the sample. Prepare a volume of master mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the master mix (control RNA was added during RNA extraction)

Volume per reaction	Volume master mix
13.8 µl Reaction Mix	13.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)

Protocol B

The control RNA is used for the control of the Real-Time-RT-PCR only (see chapter 10 "Control RNA"). In this case, prepare the master mix according to table 3.

The master mix contains all of the components needed for real RT-PCR except the sample. Prepare a volume of master mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 3: Preparation of the master mix (control RNA is added directly to the master mix)

Volume per reaction	Volume master mix
13.8 µl Reaction Mix	13.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)
0.2 µl Control RNA*	0.2 µl x (N+1)*

*The increase in volume caused by adding the control RNA is not taken into account when preparing the PCR assay.

Protocol A and B: Real-Time-RT-PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument / take an optical PCR reaction plate.
- Pipet **14 µl** of master mix into each optical PCR reaction tube.
- Add **6 µl** of the eluates from the RNA isolation (including the eluate of the water control), the respective positive control, and the negative control the corresponding optical PCR reaction tube / the optical PCR reaction plate (table 4).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the Real-Time-RT-PCR

Component	Volume
Master mix	14.0 µl
Sample	6.0 µl
Total volume	20.0 µl

11.3 Instrument settings

For the Real-Time-RT-PCR use the thermal profile shown in table 5.

Table 5: Real-Time-RT-PCR thermal profile

Description	Time	Temperature	No of cycles
Reverse Transcription	10 min	45 °C	1
Initial Denaturation	5 min	95 °C	1
Amplification of cDNA			45
Denaturation	10 s	95 °C	
Annealing and extension	40 s	60 °C	
	Aquisition at the end of this step		

Dependent on the real time instrument used, further instrument settings have to be adjusted according to table 6.

Table 6: Overview of the instrument settings required for the MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR.

Real-Time-RT-PCR Instrument	Parameter	Detection Channel	Notes
LightCycler 480II	RdRP gene / S gene Control RNA (IPC) ISC E gene	465–510 533–580 533–610 618–660	Colour Compensation Kit CoV-2 (KG19-4 CC CoV-2) required Melt factor / Quant factor / Max integration time (s) 1 / 10 / 1 1 / 10 / 2 1 / 10 / 2 1 / 10 / 3
Stratagene Mx3000P/ Mx3005P	RdRP gene / S gene Control RNA (IPC) ISC E gene	FAM HEX ROX Cy5	Gain 8 Gain 1 Gain 1 Gain 4 Reference Dye: None
ABI 7500	RdRP gene / S gene Control RNA (IPC) ISC E gene	FAM JOE ROX Cy5	Option Reference Dye ROX: NO
AriaMx Bio-Rad CFX96	RdRP gene / S gene Control RNA (IPC) ISC E gene	FAM HEX ROX Cy5	Option Reference Dye ROX: NO

Real-Time-RT-PCR Instrument	Parameter	Detection Channel	Notes
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	RdRP gene / S gene Control RNA (IPC) ISC E gene	Green Yellow Orange Red	Gain 5 Gain 5 Gain 5 Gain 5
Mic qPCR Cycler	RdRP gene / S gene Control RNA (IPC) ISC E gene	Green Yellow Orange Red	Gain 8 Gain 10 Gain 10 Gain 10

12 DATA ANALYSIS

The following results can occur (table 7):

Table 7: Interpretation reaction mix

Signal/C_T Values				Interpretation
FAM channel	**Cy5 channel**	**ROX channel**	**HEX channel**	
RdRP gene / S gene	E gene	ISC	Control RNA (IPC)	
positive	negative	positive or negative	positive or negative**	**Positive result, the sample contains SARS-CoV-2 RNA.**
positive	**positive**	positive or negative	positive or negative**	**Positive result, the sample contains SARS-CoV-2 RNA.**
negative	**positive**	positive or negative	positive or negative**	**Positive result, the sample contains SARS-CoV-2 RNA or SARS-CoV-1 RNA*.**
negative	negative	positive	≤ 34***	**Negative result, the sample contains no SARS-CoV-2 RNA and no SARS-CoV-1 RNA*.**
negative	negative	negative	≤ 34***	**No diagnostic statement can be made.** Amount or quality of sample material not sufficient.

Signal/C_T Values				Interpretation
FAM channel	**Cy5 channel**	**ROX channel**	**HEX channel**	
RdRP gene / S gene	E gene	ISC	Control RNA (IPC)	
negative	negative	positive	negative or > 34***	**Caution!** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction.
negative	negative	negative	negative or > 34***	**Caution!** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction. Amount or quality of sample material not sufficient.

* SARS-CoV-1 infections have not been reported since 2004 [5].

** A strong positive signal in the FAM, Cy5 and/or ROX can inhibit the IC. In such cases the result for the control RNA can be neglegted.

*** Depending on the PCR instrument and/or the chosen extraction method, the C_T values might be shifted. The water control can be used as reference. If the HEX C_T value of a sample differs a lot from the water control, partial inhibition has occurred, leading to false negative results in case of weak positive samples.

Figure 1, 2, 3 and 4 show examples for positive and negative real time RT-PCR results.

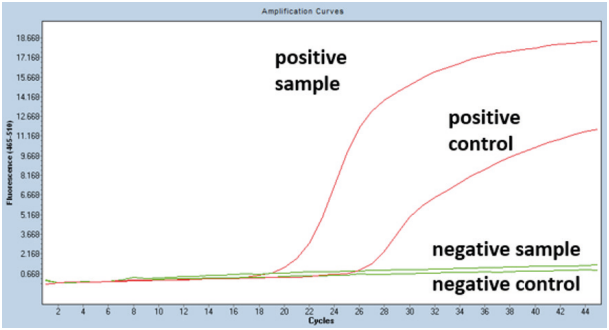

Figure 1: The positive sample shows pathogen specific amplification in the FAM channel (positive sample and positive control), whereas no fluorescence signal is detected in the negative sample or the negative control (LC480 II real time PCR instrument).

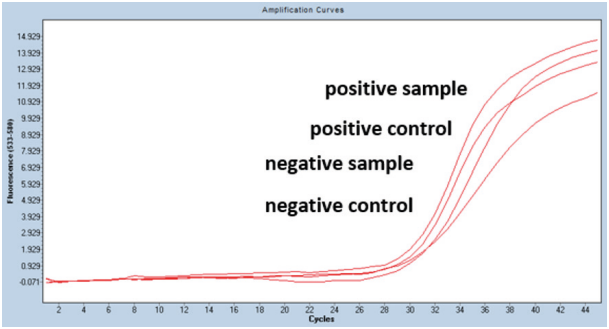

Figure 2: The positive sample, the positive control, the negative control as well as the negative sample show a signal in the control RNA-specific HEX channel (IPC). The amplification signal of the control RNA in the negative sample shows that the missing signals in the pathogen-specific channels FAM and Cy5 are not due to RT-PCR inhibition or failure of RNA isolation, but that the sample is a true negative sample (LC480 II real time PCR instrument).

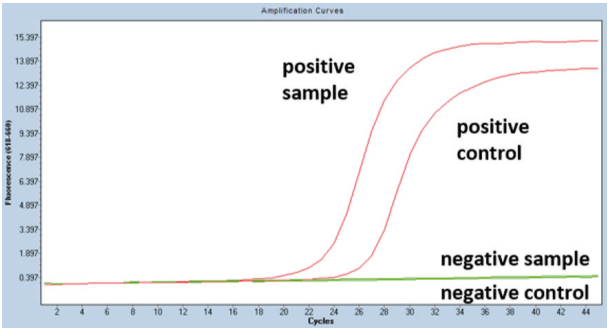

Figure 3: The positive sample shows pathogen-specific amplification in the Cy5 channel (positive sample and positive control), whereas no fluorescence signal is detected in the negative sample and the negative control (LC480 II real time PCR instrument).

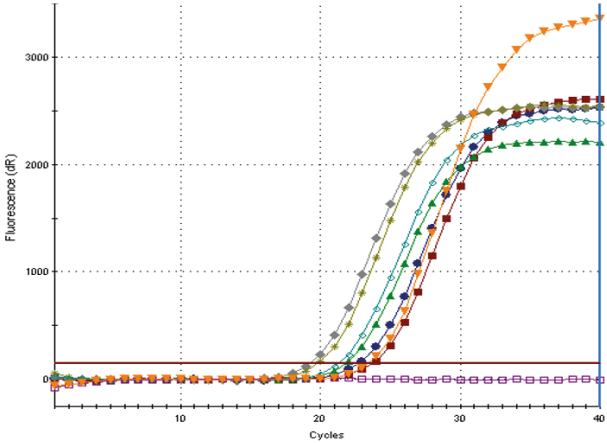

Figure 4: Signals of the amplification of the ISC in the ROX channel. The figure shows the CT values of eluates from respiratory swabs after nucleic acid extraction using MutaCLEAN® Mag Swab/ Respira extraction kit (KG1022); Stratagene Mx3005 P real time PCR instrument.

13 ASSAY VALIDATION

To increase process safety IPC is included in the negative control and positive control.

Negative controls

The negative control must show no CT in the FAM and Cy5 channel. The HEX channel (IPC) in the negative control must show a CT of below 34. Due to the high sensitivity of the MutaPLEX® Coronavirus (SARS-CoV-2) real time RT-PCR, a weak positive result in the ROX channel (ISC) caused by slight contaminations with human DNA during

RT-PCR set up cannot completely be ruled out. This does not affect the validity of the respective run (see also internal controls).

Positive controls

All the positive controls must show a positive (i. e. exponential) amplification curve in the different channels FAM, Cy5, ROX and HEX. The positive controls must fall below a C_T of 30 except for the HEX channel, which must show a C_T of below 34.

The positive control includes in vitro transcripts and synthetic DNA of approximately 10^4 copies per reaction for RdRP gene, S gene, E gene and ISC.

Internal controls

All internal controls (ISC and IPC, seqc sample and extraction quality control) must show a positive (i. e. exponential) amplification curve. The control RNA (IPC) must fall below a C_T of 34. If the control RNA is above C_T 34 ,this points to a purification problem or a strong positive sample that can inhibit the IPC. In the latter case, the assay is valid. It is recommended to perform the extraction of a water control in each run. The IPC in the water control must fall below a C_T of 34. For accurately drawn respiratory swab samples, the ISC shows C_T values from app. 15 to app. 28. A heavily delayed signal of higher than a C_T of 34 indicates a low sample amount. Therefore, false negative results cannot be ruled out. In case of no amplifications neither in the FAM nor in the Cy5 channel, there must be an amplification curve in the ROX channel (IPC) and the HEX (ISC) channel when using eluates of primary samples from multiple species such as mammals and birds.

If other nucleic acid extraction kits are used, the customer must define own cutoffs. In this case the CT value of the Control RNA (ISC) in an eluate from a sample should not be delayed for more than 4 CT in comparison to an eluate from an extracted water control.

14 LIMITATIONS OF THE METHOD

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the nCoV and Betacoronavirus genomes covered by the primers and/or probes used in the kit may result in failure to detect the respective RNA.
- As with any diagnostic test, results of the MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

15 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a Real-Time-RT-PCR.

No fluorescence signal in the FAM and Cy5 channel of the positive controls

The selected channel for analysis does not comply with the protocol

Select the FAM channel for analysis of the nCoV specific amplification, the Cy5 channel for analysis of the betacoronavirus specific amplification, the HEX channel for the amplification of the control RNA and the ROX channel for the amplification of the ISC.

Incorrect preparation of the Master Mix

Make sure the enzyme is added to the master mix (chapter 11).

Incorrect configuration of the Real-Time-RT-PCR

Check your work steps and compare with chapter "Procedure".

The programming of the thermal profile is incorrect

Compare the thermal profile with the protocol (table 5).

Incorrect storage conditions for one or more kit components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter "Transport, storage and stability".

Weak or no signal of the control RNA and simultaneous absence of a signal in the virus-specific FAM and/or Cy5 channel

Real-Time-RT-PCR conditions do not comply with the protocol

Check the Real-Time-RT-PCR conditions (chapter 11).

Real-Time-RT-PCR inhibited

Make sure that you use an appropriate isolation method (see "Sample preparation") and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffer of the isolation kit has been completely removed.

Sample material not sufficient

Make sure enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter "Sample preparation") and follow the manufacturer's instructions

RNA loss during isolation process

In case the control RNA was added before extraction, the lack of an amplification signal can indicate that the RNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.

Incorrect storage conditions for one or more components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter "Transport, storage and stability".

Detection of a fluorescence signal in the FAM and/or Cy5 channel of the negative control

Contamination during preparation of the RT-PCR

Repeat the Real-Time-RT-PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the positive control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the Real-Time-RT-PCR.

Detection of a fluorescence signal in the ROX channel of the negative control

Contamination with human DNA during preparation of the real time RT-PCR

As long as the ROX channel shows very high CT values, the contamination is negligible.

If the FAM and Cy5 channel are negative in the negative control, the PCR is still valid for the detection of SARS-CoV-2.

16 KIT PERFORMANCE

16.1 Analytical sensitivity

The limit of detection (LoD) of MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit was determined using serial dilutions of synthetic RNA-fragments containing the nCoV target sequence and the Betacoronavirus target sequence in a Stratagene Mx3005 real time PCR instrument. The LoD of MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit is ≤10 genome copies per reaction each.

16.2 Analytical specificity

The specificity of the MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit was evaluated with different other relevant viruses and bacteria found in clinical samples and basing on in silico analyses.

The MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit showed a positive result for the samples containing SARS-CoV-2 and Betacoronavirus RNA sequences, whereas samples containing other pathogens were reliably tested negative. The results are shown in table 8.

Table 8: Eluted DNA and RNA from bacterial and viral pathogens tested for the determination of MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR.

Eluates with known status	**Expected result E gene**	**Expected result RdRP gene / S gene**	**MutaPLEX® Coronavirus E gene**	**MutaPLEX® Coronavirus RdRP gene / S gene**
	Cy5 channel	**FAM channel**	**Cy5 channel**	**FAM channel**
SARS-CoV-2	*positive*	*positive*	*positive*	*positive*
SARS-CoV-1	*positive*	negative	*positive*	negative
MERS-CoV	negative	negative	negative	negative

Eluates with known status	Expected result E gene	Expected result RdRP gene / S gene	MutaPLEX® Coronavirus E gene	MutaPLEX® Coronavirus RdRP gene / S gene
	Cy5 channel	FAM channel	Cy5 channel	FAM channel
HCoV-229E	negative	negative	negative	negative
HCoV-0C43	negative	negative	negative	negative
Influenza A H3N2	negative	negative	negative	negative
Influenza A H5N1	negative	negative	negative	negative
Influenzavirus B	negative	negative	negative	negative
Respiratory Syncytial Virus A	negative	negative	negative	negative
Respiratory Syncytial Virus B	negative	negative	negative	negative
Parainfluenza-virus 1	negative	negative	negative	negative
Parainfluenza-virus 2	negative	negative	negative	negative
Parainfluenza-virus 3	negative	negative	negative	negative
Parainfluenza-virus 4	negative	negative	negative	negative
Metapneumo-virus	negative	negative	negative	negative
Adenovirus	negative	negative	negative	negative
Rhinoviruses	negative	negative	negative	negative
Enteroviruses	negative	negative	negative	negative
Human Bocavirus	negative	negative	negative	negative

Eluates with known status	Expected result E gene	Expected result RdRP gene / S gene	MutaPLEX® Coronavirus E gene	MutaPLEX® Coronavirus RdRP gene / S gene
	Cy5 channel	FAM channel	Cy5 channel	FAM channel
Legionella pneumophila	negative	negative	negative	negative
Mycoplasma pneumophila	negative	negative	negative	negative
Mycobacterium tuberculosis complex	negative	negative	negative	negative
Bordetella pertussis	negative	negative	negative	negative
Bordetella parapertussis	negative	negative	negative	negative
S. aureus	negative	negative	negative	negative
MRSA	negative	negative	negative	negative
MSSA	negative	negative	negative	negative
Streptococcus spp.	negative	negative	negative	negative

16.3 Clinical samples

Positive (50) and negative (153) confirmed samples (oral and nasal swabs) from the pandemic outbreak 2020 in Europe were tested.

The RNA was extracted by using the MutaCLEAN® Complete Mag RNA/DNA (KG1020) extraction kit on a KingFisher Prime Duo Instrument.

The PCR experiments were performed on a MX3005p Stratagene Cycler. The retesting of the confirmed samples with MutaPLEX® Coronavirus (SARS-CoV-2) showed a sensitivity of 100 % and a specificity of 100 %. None of the samples were inhibited in the PCR.

	Positive samples	Negative samples
MutaPLEX® Coronavirus positive	50	0
MutaPLEX® Coronavirus negative	0	153
	Sensitivity [%]	**Specificity [%]**
	100	100

16.4 Linear range

The linear range of the MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit was evaluated by analysing logarithmic dilution series of in vitro transcripts and synthetic DNA fragments.

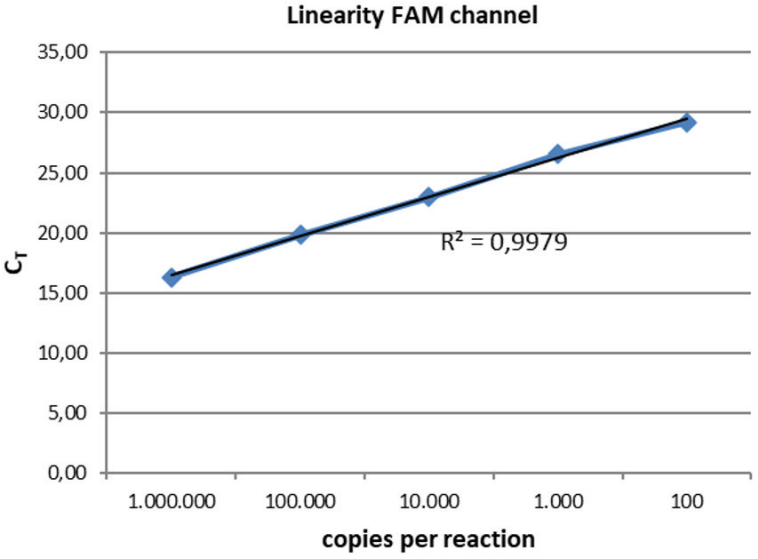

Figure 5: Determination of the linear range of MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR in the FAM channel.

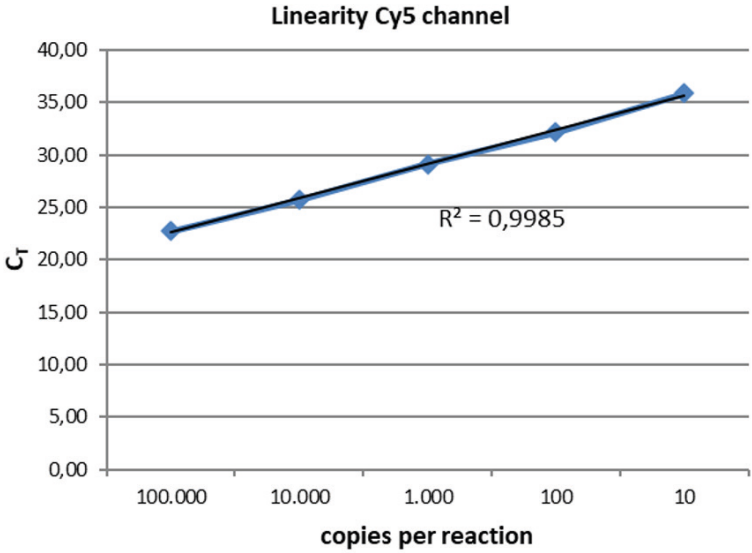

Figure 6: Determination of the linear range of MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR in the Cy5 channel.

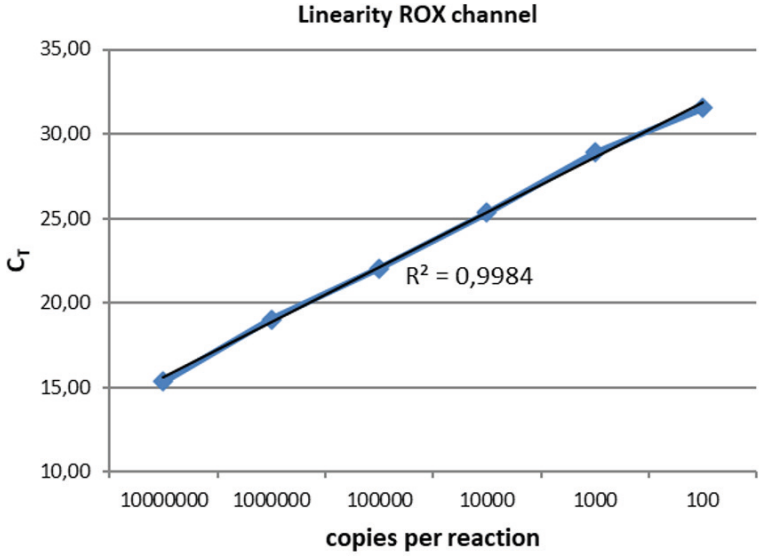

Figure 7: Determination of the linear range of MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR in the ROX channel.

16.5 Precision

The precision of the MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit was determined as intra-assay variability, inter-assay variability and inter-lot variability.

Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of RdRP gene in vitro transcripts and E gene in vitro transcripts, ISC-specific DNA and on the threshold cycle of the control RNA (IPC).

Table 8: Precision of the MutaPLEX® Coronavirus (SARS-CoV-2) Real-Time-RT-PCR Kit.

RdRP gene and S gene (FAM)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.23	0.77
Inter-Assay-Variability	25	0.51	1.71
Inter-Lot-Variability	25	0.76	2.56

E gene (Cy5)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.27	0.84
Inter-Assay-Variability	25	0.51	1.50
Inter-Lot-Variability	25	0.52	1.61

ISC (ROX)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.28	0.90
Inter-Assay-Variability	25	0.40	1.27
Inter-Lot-Variability	25	0.25	0.77

IPC (HEX)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.69	2.31
Inter-Assay-Variability	25	0.58	1.91
Inter-Lot-Variability	25	0.37	1.22

16.6 Diagnostic Sensitivity

The diagnostic sensitivity of Real-Time-RT-PCR assays is mainly dependent on the DNA/RNA extraction method used to isolate DNA and RNA from various biological specimens. DNA/RNA extraction reagents are not part of the Immundiagnostik AG

Real-Time-RT-PCR kits. Immundiagnostik AG Real-Time-RT-PCR kits include an extraction control and guidelines for the validation criteria of the extraction control in each reaction. The extraction control indicates inhibition of the Real-Time-RT-PCR and/or inefficient nucleic acid extraction. It cannot be used as a calibrator.

Therefore, Immundiagnostik AG guarantees the analytical sensitivities and specificities of the real time RT- PCR kits, performed with eluted DNA and RNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. Immundiagnostik AG does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of Immundiagnostik AG Real-Time-RT-PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods.

It is the responsibility of the user to qualify the extraction methods used for DNA/RNA isolation from biological samples.

17 ABBREVIATIONS AND SYMBOLS

(c)DNA	(complementary) Deoxyribonucleid acid	REF	Catalog number
RNA	Ribonucleid acid	→REF	To be used with
PCR	Polymerase chain reaction	Σ	Contains sufficient for <n> test
RT	Reverse transcription		Upper limit of temperature
RT-PCR	Reverse transcription-PCR		Manufacturer
REACTION MIX	Reaction mix		Use by
ENZYME	Enzyme	LOT	Lot number
CONTROL +	Positive control	CONT	Content
CONTROL –	Negative control		Consult instructions for use
CONTROL RNA IPC	Control RNA (IPC)	IVD	*In vitro* diagnostic medical device

18 LITERATURE

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4. https://www.ncbi.nlm.nih.gov/research/coronavirus/
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