

MutaPLEX® RespiraScreen 1

Real-Time-RT-PCR-Kit

For the simultaneous in vitro detection and differentiation of RNA of Influenza Virus (Flu A and Flu B), Respiratory Syncytial Virus (RSV A and RSV B) and SARS-CoV-2, extracted from biological specimens.

Valid from 2020-06-30

 **KG192796**
KG1927-384


96/384

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 IVD

 CE



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1 INTENDED USE

The MutaPLEX® RespiraScreen 1 real time RT-PCR Kit is a screening assay for the simultaneous detection of three groups of different respiratory viruses. The design allows the differentiation of Influenza Virus (Flu A, Flu B) and Respiratory Syncytial Virus (RSV A, RSV B) from the pandemic coronavirus SARS-CoV-2, extracted from biological specimens.

2 PATHOGEN INFORMATION

Influenza viruses belong to the family of Orthomyxoviridae and are the causative agent of 'the flu'. Influenza A and B viruses have a single stranded RNA genome, consisting of 8 RNA segments. The genome of Influenza A viruses is characterised by a high mutation frequency, the so-called "antigenic drift". Numerous subtypes of Influenza A viruses are known. They can be categorised by their surface antigens H (haemagglutinin) and N (neuraminidase): Influenza A (H1N1) Virus, Influenza A (H5N1) Virus etc. Therefore, yearly in silico analysis of the sequences of newly emerged subtypes is done, to prevent false negative results caused by primer and/or probe mismatches. Influenza B viruses show a 2–3 times slower mutation rate than type A.

Respiratory syncytial viruses are enveloped negative-sense, single stranded RNA viruses of the Pneumoviridae family, genus *Orthopneumovirus*. RSV are divided into subgroups A and B. RSV is a virus that causes infections of the lungs and respiratory tract. It is so common that most children have been infected with the virus at the age of 2. RSV can also infect adults.

In adults and older, healthy children, the symptoms of RSV infections are mild and typically mimic the common cold. Self-care measures are usually all that is needed to relieve any discomfort. Infection with RSV can be severe in some cases, especially in premature babies and infants with underlying health conditions. RSV can also become serious in older adults, adults with heart and lung diseases, or anyone with a very weak immune system (immunocompromised).

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) and Severe Acute Respiratory Syndrome (SARS). The novel Coronavirus (SARS-CoV-2) is a new strain within the Sarbecoviruses 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® RespiraScreen 1 real time RT-PCR Kit contains specific primers and dual-labelled probes for the amplification of RNA (cDNA) of Influenza A (M gene), Influenza B (NEP gene), RSV A (G gene), RSV B (G gene) and SARS-CoV-2 (E gene) extracted from biological specimen.

Furthermore, MutaPLEX® RespiraScreen 1 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-labelled 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.

4 PACKAGE CONTENTS

The reagents supplied are sufficient for 96 (KG192796) or 384 (KG1927-384) reactions, respectively.

Table 1: Components of the MutaPLEX® RespiraScreen 1 Real-Time-RT-PCR Kit .

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

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- RNA isolation kit (e.g. MutaCLEAN® Mag RNA/DNA, KG1023)
- 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® RespiraScreen 1 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. Up to 20 freeze and thaw cycles are possible.

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® RespiraScreen 1 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® RespiraScreen 1 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® Mag RNA/DNA (KG1023) 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.

Control RNA used as extraction control

MutaPLEX® RespiraScreen 1 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.

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

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 temperature for 15 min is recommended.

11.2 Procedure

If the control RNA is used to control both, the Real-Time-RT-PCR and the RNA isolation procedure, please work as described in the following protocol.

Protocol

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)

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 / the optical PCR reaction plate.
- 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 3).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 3: 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 4.

Table 4: 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 5.

Table 5: Overview of the instrument settings required for the MutaPLEX® RespiraScreen 1 Real-Time-RT-PCR.

Real-Time-RT-PCR Instrument	Parameter	Detection Channel	Notes		
LightCycler 480II	Flu A / Flu B Control RNA (IPC) SARS-CoV-2 RSV A / RSV B	465–510 533–580 533–610 618–660	Color compensation kit MutaPLEX® CC-1 (KG19-5-CC) required		
			Melt factor	Quant factor	Max integration time (s)
			1	10	1
			1	10	2
			1	10	2
1	10	3			
Stratagene Mx3000P/ Mx3005P	Flu A / Flu B Control RNA (IPC) SARS-CoV-2 RSV A / RSV B	FAM HEX ROX Cy5	Gain 8	Reference Dye: None	
			Gain 1		
			Gain 1		
			Gain 4		
ABI 7500	Flu A / Flu B Control RNA (IPC) SARS-CoV-2 RSV A / RSV B	FAM JOE ROX Cy5	Reference Dye: None		

Real-Time-RT-PCR Instrument	Parameter	Detection Channel	Notes
AriaMx Bio-Rad CFX96	Flu A / Flu B Control RNA (IPC) SARS-CoV-2 RSV A / RSV B	FAM HEX ROX Cy5	Reference Dye: None
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	Flu A / Flu B Control RNA (IPC) SARS-CoV-2 RSV A / RSV B	Green Yellow Orange Red	Gain 5 Gain 5 Gain 5 Gain 5
Mic qPCR Cyclcr	Flu A / Flu B Control RNA (IPC) SARS-CoV-2 RSV A / RSV B	Green Yellow Orange Red	Gain 8 Gain 10 Gain 10 Gain 10

12 DATA ANALYSIS

The following results can occur:

Signal/C _T Values				Interpretation
FAM channel	ROX channel	Cy5 channel	HEX channel	
Flu A / Flu B	SARS-CoV-2	RSV A / RSV B	Control RNA (IPC)	
positive³	positive⁴	positive	positive or negative ¹	Positive result. The sample contains RNA of Flu A and/or Flu B, RSV A and/or RSV B and SARS-CoV-2.
positive³	positive⁴	negative	positive or negative ¹	Positive result. The sample contains RNA of Flu A and/or Flu B and SARS-CoV-2.
positive³	negative	negative	positive or negative ¹	Positive result. The sample contains RNA of Flu A and/or Flu B.

Signal/C _T Values				Interpretation
FAM channel	ROX channel	Cy5 channel	HEX channel	
Flu A / Flu B	SARS-CoV-2	RSV A / RSV B	Control RNA (IPC)	
negative	positive ⁴	positive	positive or negative ¹	Positive result. The sample contains RNA of RSV A and/or RSV B and SARS-CoV-2.
negative	positive ⁴	negative	positive or negative ¹	Positive result. The sample contains RNA of SARS-CoV-2.
negative	negative	positive	positive or negative ¹	Positive result. The sample contains RNA of RSV A and/or RSV B.
negative	negative	negative	≤ 34	Negative result. The sample contains no RNA of Flu A and/or Flu B, RSV A and/or RSV B or SARS-CoV-2.
negative	negative	negative	negative or > 34 ²	Caution! The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction.

¹ A strong positive signal in the FAM, Cy5 or ROX channel can inhibit the IPC. In such cases the result for the Control RNA can be neglected.

² In case of high C_T values, the IPC should be compared to the water extraction control as described in the chapter 'Assay validation'.

³ A differentiation between Flu A and Flu B is possible with a second PCR (e.g. MutaPLEX® RespiroSys1, KG198496).

⁴ The WHO recommends the detection of 2 target genes in regions with low SARS-CoV-2 prevalence. The result should be confirmed with another PCR (e.g. MutaPLEX® Coronavirus, KG192696). In high prevalence regions for SARS-CoV-2, a single target is sufficient for the SARS-CoV-2 detection [4].

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

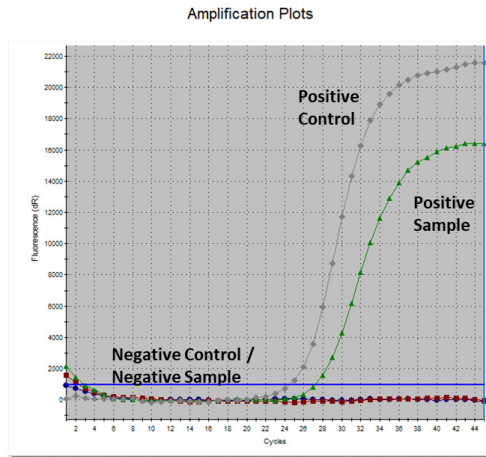


Figure 1: The positive sample shows pathogen specific amplification in the FAM channel (positive FLU sample and positive control), whereas no fluorescence signal is detected in the negative sample or the negative control (Mx3005P qPCR System).

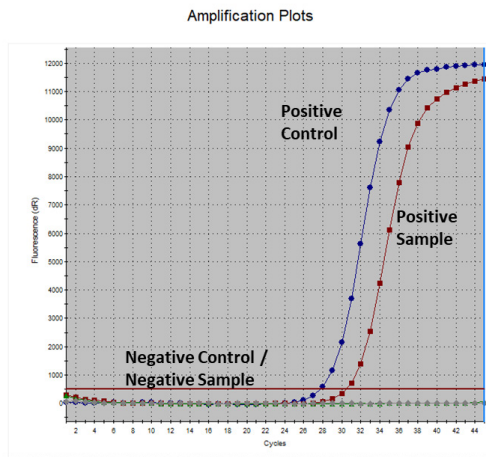


Figure 2: The positive sample shows pathogen specific amplification in the ROX channel (positive SARS-CoV-2 sample and Positive Control), whereas no fluorescence signal is detected in the negative sample and the Negative Control (Mx3005P qPCR System).

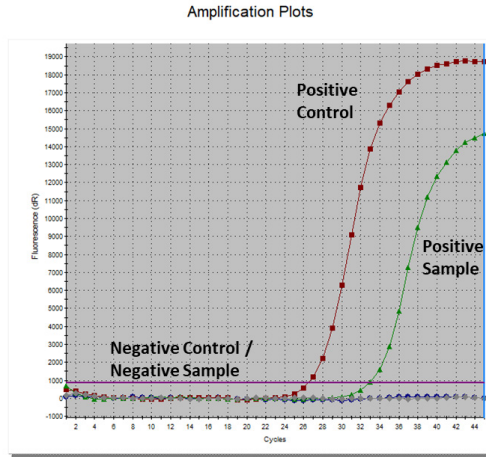


Figure 3: Figure 3: The positive sample shows pathogen specific amplification in the Cy5 channel (positive RSV sample and Positive Control), whereas no fluorescence signal is detected in the negative sample and the Negative Control (Mx3005P qPCR System).

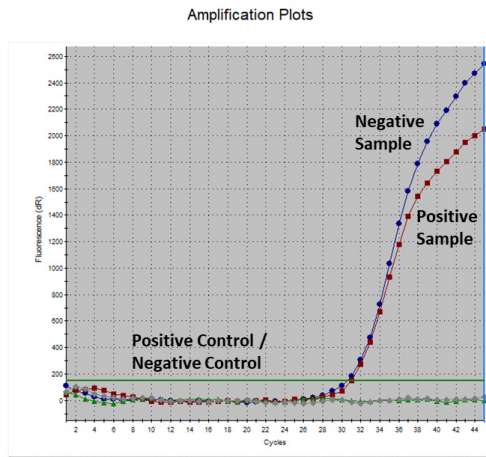


Figure 4: The positive sample and the negative sample show an amplification curve, whereas the Positive Control and the Negative Control don't show a signal in the Control RNA specific HEX channel (Mx3005P qPCR System).

13 ASSAY VALIDATION

Negative control

The negative control must show no C_T in the FAM, HEX, ROX and Cy5 channel.

Positive controls

All parameters in the positive control must show a positive (i.e. exponential) amplification curve in the different channels FAM, Cy5 and ROX. The positive controls must fall below a C_T of 30. The positive control includes in vitro transcripts of Flu A (M gene), Flu B (NEP gene), RSV A (G gene) RSV B (G gene) and SARS-CoV-2 (E gene).

Internal controls

The following values for the amplification of the internal controls are valid using nucleic acid extraction kits MutaCLEAN® Mag RNA/DNA or MutaCLEAN® Universal RNA/DNA. The control RNA (IPC) must show a positive (i.e. exponential) amplification curve and 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.

If other nucleic acid extraction kits are used, the customer must define own cut-offs. In this case the C_T value of the control RNA (IPC) in an eluate from a sample should not be delayed for more than 4 C_T 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 Flu A, Flu B, RSV A, RSV B and SARS-CoV-2 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® RespiraScreen 1 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.

If you have further questions, please do not hesitate to contact our scientists on info@immundiagnostik.com.

No fluorescence signal in the FAM and/or ROX and/or 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 Flu specific amplification, the ROX channel for analysis of the SARS-CoV-2 specific amplification, the HEX channel for the amplification of the control RNA and the Cy5 channel for the amplification of the RSV.

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 4).

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 FAM and/or ROX 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 chapter “Sample preparation”) and follow the manufacturer’s instructions. Make sure that the ethanol-containing buffers have 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 ROX and/or Cy5 and/or HEX channel of the negative control

Contamination during preparation of the real-time RT-PCR

Repeat the Real-Time-RT-PCR in replicates. If the result is negative in the repetition, the contamination occurred 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.

16 KIT PERFORMANCE

16.1 Analytical sensitivity

The limit of detection (LoD) of MutaPLEX® RespiraScreen 1 real-time RT-PCR kit was determined testing serial dilutions of synthetic RNA-fragments containing the specific gene target sequence on a Stratagene Mx3005P real time PCR instrument. The estimated LoD of MutaPLEX® RespiraScreen 1 real time RT-PCR Kit is ≤ 10 genome copies per reaction for each parameter (Flu A, Flu B, SARS-CoV-2, RSV A, RSV B).

16.2 Analytical specificity

The specificity of the MutaPLEX® RespiraScreen 1 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 results for the wet analysis are shown in table 6, the results for the insilico analysis are shown in table 7.

The primers and probes for SARS-CoV-2 may detect SARS-CoV-1 as well, but since there is no report on SARS-CoV-1 cases since 2004, it is very unlikely to happen [5].

Table 6: Eluted RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of MutaPLEX® RespiroScreen 1 real time RT-PCR kit.

Eluates with known status	Result Flu A/ Flu B	Result SARS-CoV-2	Result RSV A/ RSV B	Result IPC
	FAM channel	ROX channel	Cy5 channel	HEX channel
Parainfluenzavirus 1	negative	negative	negative	positive
Parainfluenzavirus 2	negative	negative	negative	positive
Parainfluenzavirus 3	negative	negative	negative	positive
Parainfluenzavirus 4	negative	negative	negative	positive
Metapneumovirus	negative	negative	negative	positive
Adenovirus	negative	negative	negative	positive
Rhinovirus	negative	negative	negative	positive
Enterovirus	negative	negative	negative	positive
Human Bocavirus	negative	negative	negative	positive
Legionella pneumophila	negative	negative	negative	positive
Mycoplasma pneumophila	negative	negative	negative	positive
Mycobacterium tuberculosis complex	negative	negative	negative	positive
Bordetella pertussis	negative	negative	negative	positive
Bordetella parapertussis	negative	negative	negative	positive
Staphylococcus aureus	negative	negative	negative	positive
MRSA	negative	negative	negative	positive
MSSA	negative	negative	negative	positive
Streptococcus spp.	negative	negative	negative	positive
SARS-CoV-1	negative	positive	negative	positive
MERS-CoV	negative	negative	negative	positive
SARS-CoV-2	negative	positive	negative	positive

Eluates with known status	Result Flu A/ Flu B	Result SARS-CoV-2	Result RSV A/ RSV B	Result IPC
	FAM channel	ROX channel	Cy5 channel	HEX channel
Influenzavirus A H1N1	positive	negative	negative	positive
Influenzavirus A H3N3	positive	negative	negative	positive
Influenzavirus A H5N1	positive	negative	negative	positive
Influenzavirus B	positive	negative	negative	positive
Respiratory Syncytial Virus A	negative	negative	positive	positive
Respiratory Syncytial Virus B	negative	negative	positive	positive

Table 7: Inclusivity of the MutaPLEX® RespiraScreen 1 real time RT-PCR kit primers and probes (in silico analysis).

12–5000 whole genome sequences		Homology	Comment
NCBI / GISAID	SARS-CoV-2	Forward primer	2319 sequences: 100% 1 sequence: 96% (1 mismatch)
		Reverse primer	2318 sequences: 100% 2 sequences: 95% (1 mismatch)
		Probe	2317 sequences: 100% 3 sequences: 96% (1 mismatch)
NCBI	RSV A / RSV B	Forward primer	12 sequences: 100% no mismatch
		Reverse primer	2 sequences: 100% 10 sequences: 96% (1 mismatch)
		Probe	10 sequences: 100% 2 sequences: 96% (1 mismatch)

12–5000 whole genome sequences		Homology	Comment	
NCBI	Flu B	Forward primer	1000 sequences: 100%	no mismatch
		Reverse primer	1000 sequences: 100%	no mismatch
		Probe	998 sequences: 100%	2 sequences: 96% (1 mismatch)
NCBI	Flu A	Forward primer	5000 sequences: 100%	no mismatch
		Reverse primer	5000 sequences: 100%	no mismatch
		Probe	5000 sequences: 100%	no mismatch

16.3 Clinical samples

Positive (35) and negative (173) confirmed samples (oral and nasal swabs) from the pandemic COVID-19 outbreak 2020 in Europe were tested.

The RNA was extracted by using the MutaCLEAN® Mag RNA/DNA (KG1023) extraction kit on a KingFisher Prime Duo Instrument.

The PCR experiments were performed on a Mx3005P Stratagene Cycler. The testing of the confirmed samples with MutaPLEX® RespiraScreen 1 real time RT-PCR kit showed a sensitivity of 100 % and a specificity of 100 %. None of the samples were inhibited in the real time RT-PCR. For the validation of the MutaPLEX® RespiraScreen 1 real time RT-PCR kit the eluates of all samples were retested and showed the same results.

	SARS-CoV-2 positive samples	SARS-CoV-2 negative samples
MutaPLEX® RespiraScreen 1 positive SARS-CoV-2	35	0
MutaPLEX® RespiraScreen 1 negative SARS-CoV-2	0	172
	Sensitivity [%]	Specificity [%]
	100	100

16.4 Linear range

The linear range of the MutaPLEX® RespiraScreen 1 real-time RT-PCR kit was evaluated by analysing logarithmic dilution series of in vitro transcripts and synthetic DNA fragments.

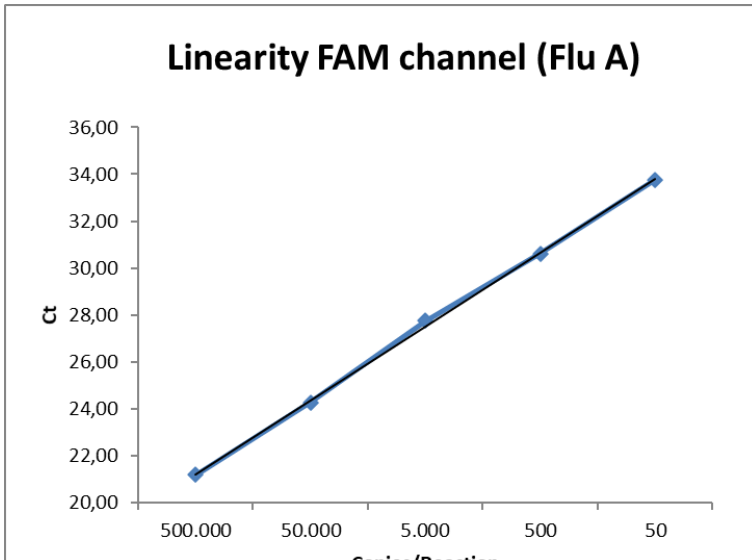


Figure 5: Determination of the linear range of MutaPLEX® RespiraScreen 1 real time RT-PCR Kit for Flu A in the FAM channel.

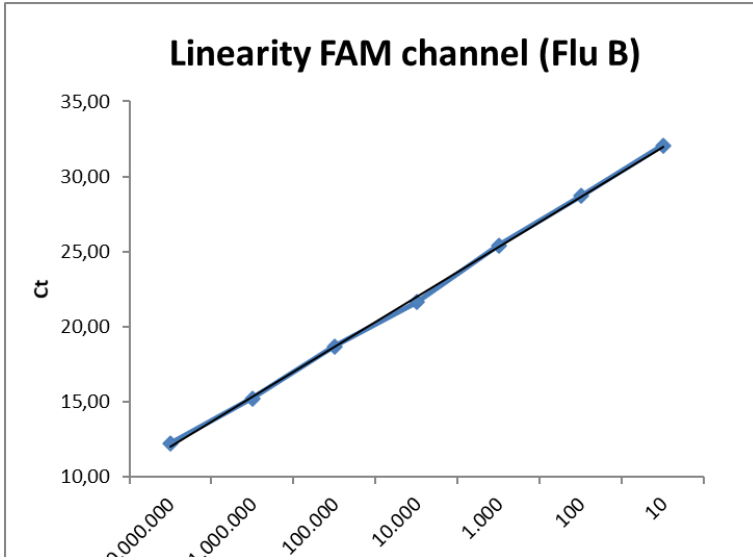


Figure 6: Determination of the linear range of MutaPLEX® RespiraScreen 1 real time RT-PCR kit for Flu B in the FAM channel.

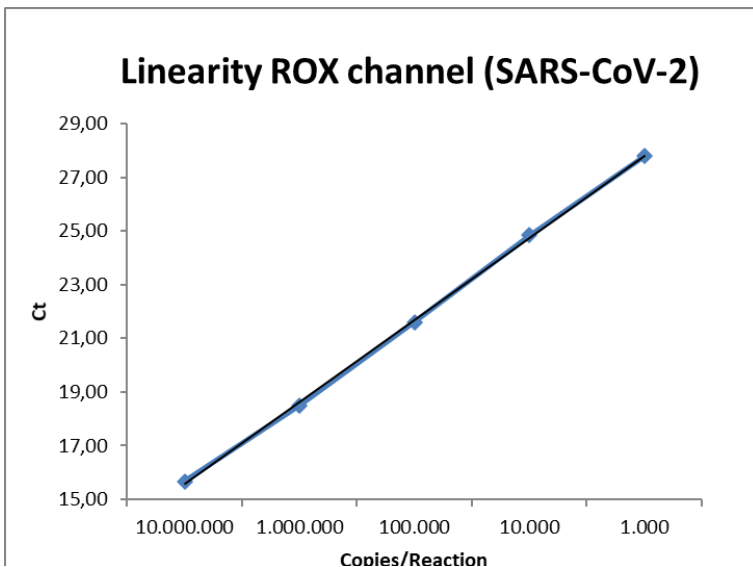


Figure 7: Determination of the linear range of MutaPLEX® RespiraScreen 1 real time RT-PCR kit for SARS-CoV-2 in the ROX channel.

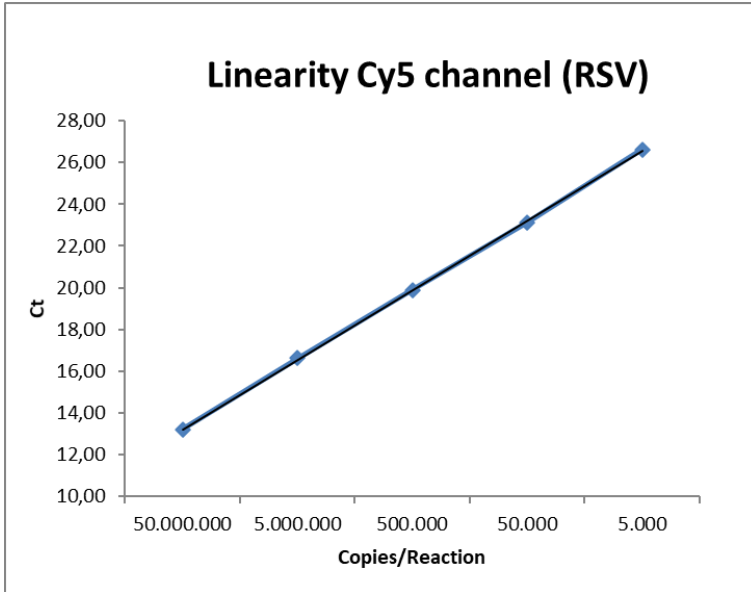


Figure 8: Determination of the linear range of MutaPLEX® RespiraScreen 1 real time RT-PCR Kit for RSV A and RSV B in the Cy5 channel.

16.5 Precision

The precision of the MutaPLEX® RespiraScreen 1 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 M gene (Flu A) in vitro transcripts, NEP gene (Flu B) in vitro transcripts, E gene (SARS-CoV-2) in vitro transcripts, G gene in vitro transcripts (RSV) and on the threshold cycle of the Control RNA (IPC). The results are shown in table 8.

Table 8: Precision of the MutaPLEX® RespiraScreen 1 real time RT-PCR kit.

Flu A, M gene (FAM)	copies/ reaction	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	50	0.30	0.88
Inter-Assay-Variability	50	0.22	0.65
Inter-Lot-Variability	50	0.22	0.65

Flu B, NEP gene (FAM)	copies/ reaction	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	10	0.28	0.89
Inter-Assay-Variability	10	0.26	0.80
Inter-Lot-Variability	10	0.25	0.79

SASR-CoV-2, E gene (ROX)	copies/ reaction	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	10	0.33	0.98
Inter-Assay-Variability	10	0.11	0.34
Inter-Lot-Variability	10	0.04	0.11

RSV A / RSV B, G gene (Cy5)	copies/ reaction	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	50	0.31	0.91
Inter-Assay-Variability	50	0.39	1.15
Inter-Lot-Variability	50	0.24	0.71

IPC (HEX)	copies/ reaction	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	1000	0.47	1.60
Inter-Assay-Variability	1000	0.48	1.61
Inter-Lot-Variability	1000	0.25	0.86






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 real time (RT-)PCR kits. Immundiagnostik 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 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 does not guarantee diagnostic sensitivities. If diagnostic sensitivities are

mentioned in manuals of Immundiagnostik 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

RNA	Ribonucleid acid		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	<i>In vitro</i> diagnostic medical device
	Contains sufficient for <n> test	→REF	To be used with
REF	Catalog number		

18 LITERATURE

1. www.who.int/health-topics/coronavirus
2. Corman et al. Detection of 2019 novel coronavirus (2019-nCoV) by real time RT-PCR. Eurosurveillance, Volume 25, Issue 3, 23/Jan/2020.
3. www.nature.com/articles/s41564-020-0695-z, 02/March/2020
4. <https://www.who.int/publications/i/item/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117>
5. <https://www.nhs.uk/conditions/sars/>