Instructions for Use

In-vitro-Diagnostic IVD

The res4plex *direct* RT-PCR is a real-time RT-PCR test for simultaneous in vitro detection and differentiation of RNA from **SARS-CoV-2**, **Flu A**, **Flu B**, and **RSV** (RSV A and RSV B).

The res4plex *direct* RT-PCR test was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™.
In general, *direct* PCR tests are compatible with other qPCR cycler (e.g., MIC Cycler, AJ qTOWER, LightCycler® 96 or 480 I).

Content

1. Intended Use

The res4plex *direct* RT-PCR test is an in vitro real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of viral RNA of the pathogens SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV A and RSV B) from respiratory tract specimens (nasopharyngeal or oropharyngeal swabs) from individuals suspected of having a serious viral cold. The results support the differential diagnosis of infections with SARS-CoV-2, Influenza viruses and RSV. The test is intended for use in qualified laboratories by personnel trained in molecular diagnostic techniques.

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 characterized by a high mutation frequency, the so-called 'antigenic drift'. Numerous subtypes of Influenza A Viruses are known. They can be categorized by their surface antigens H (haemagglutinin) and N (neuraminidase): Influenza A (H1N1) Virus, Influenza A (H5N1) Virus etc.[1]

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. In adults and older, healthy children, the symptoms of RSV infections are mild and typically mimic the common cold. 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).[2]

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 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.[3]

3. Testing Principle

The res4plex *direct* RT-PCR test is designed to detect SARS-CoV-2, Influenza A, B and RSV either directly from the swab or after RNA extraction from patient samples on a commercially available qPCR cycler. It consists of a test kit with solutions that contain all necessary chemicals for RT-PCR (buffer components, substances for reverse transcription and PCR including an internal process control). After adding extracted RNA or untreated patient sample to the reaction solution, the RT-PCR analysis is carried out directly in the qPCR device.

The res4plex *direct* RT-PCR test contains probes specific for the following targets: (1) SARS-CoV-2 (N gene and E gene), (2) Influenza A (MP gene), (3) Influenza B (NS gene), (4) RSV (RSV A and RSV B, N gene) and (5) Internal Control. The probes are each labelled with fluorescent reporter dyes and a second dye that serves as a quencher and suppresses the fluorescence signals of the intact probes. The analysis is performed by determining 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 Ct value. The Internal Control (IC) allows the detection of RT-PCR inhibition and may also act as control for nucleic acid isolation from the biological specimen. 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.

[1] 16 May 2022, https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Influenza_saisonal.html;jsessionid=0D4B917F987
[2] 16 May 2022, https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_RSV.html
[3] 16 May 2022, https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Virologische_Basisdaten.html

4. Package Content

The reagents of each vial set are sufficient for 96 reactions. Each set contains:

Material	Lid Colour	Comment
Solution A	blue	Reaction buffer, enzymes, primer and probes
Solution B	yellow	Internal Control (artificial RNA target)
Negative Control	colourless	RNA/DNA-free molecular grade water
Positive Control	green	RNA of SARS-CoV-2, Flu A, Flu B and RSV

Additionally, a quick start protocol is provided per packing unit.

5. Additional Equipment and Reagents

- qPCR cycler
- disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- Pipette tips with filter (DNase/RNase-free)
- Table centrifuge
- RNA isolation kit (for procedure with RNA extraction, only)

6. Transport, Storage and Stability

The res4plex *direct* RT-PCR test is shipped on dry ice. All components must be stored at -25 °C to -18 °C in the dark immediately after receipt. Reagents should be handled at +2 °C to +8 °C and used within 4 hours. Repeated thawing and freezing of the components – more than once – shall be avoided. If necessary, aliquoting of the test components in RNase-free vials after the first thawing is recommended. The package bears an expiry date, after which no quality guarantee can be given.

7. Warnings and Safety Precautions

The res4plex *direct* RT-PCR test is intended for in vitro diagnostic use only. The test should only be performed by personnel trained in molecular diagnostic techniques. 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.

- 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.
- All patient samples must be treated as potentially infectious material.
- Discard sample and assay waste (that was in contact with patient material) according to your local, regional or national safety regulations.
- The concentration specifications and incubation times of the manufacturers must be followed.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging.
- Protect reagents from heat, moisture, and light.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Avoid contamination of the test by microorganisms and nucleases (DNases and RNases).
- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- Good laboratory practice should be followed during the test.
- 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.
- 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.

8. Important Points before Starting/Sample Material

Follow the procedures in these instructions for use: reliable results can only be guaranteed if proper procedures are used for sampling (naso- or oropharyngeal swabs according to manufacturer's instructions), transportation, storage, and handling of the samples.

This test is intended for the detection of SARS-CoV-2, Influenza A, Influenza B, and RSV RNA in nasopharyngeal and oropharyngeal swabs. Other specimen types may cause false negative or invalid results.

The presence of various interfering substances can lead to false negative or invalid results. The res4plex *direct* RT-PCR test includes an internal control (artificial RNA target) to detect samples containing substances that interfere with RNA isolation, reverse transcription and PCR amplification.

In every RT-PCR run one Positive Control and one Negative Control should be included. The Positive Control FBC107-PC consists of RNA of SARS-CoV-2, Flu A, Flu B and RSV. A failed positive or Negative Control will invalidate the RT-PCR run and the results must not be reported.

If you intend to analyse isolated RNA, please follow the "Procedure with RNA extraction". In this case, the starting material for the res4plex *direct* RT-PCR test is RNA isolated from biological specimens (respiratory samples). This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods must be conducted prior to using this assay. RNA extraction reagents are not part of the res4plex *direct* RT-PCR test.

If you intend to directly analyse solubilised patient samples without prior RNA extraction, please follow the "Procedure for direct testing". In that case, the solubilized patient sample is directly used for RT-PCR (without prior RNA isolation). For direct testing, the following solubilisations or viral/universal transport media have been validated as suitable:

- 0.9% NaCl solution (with or without 5 mM TCEP),
- 0.45% NaCl solution,
- molecular grade water,
- Sigma-Virocult® (mwe medical wire),
- Amies solution,
- UTM (Copan),
- Yocon VTM/UTM,
- BD™ Universal Viral Transport System,
- IsoFlow Sheath Fluid (Beckmann Coulter), and
- PBS buffer

Patient samples in solutions with chaotropic salts (such as guanidinium thiocyanate), Cobas or Hologic PCR-Medium must not be used for direct testing without prior RNA isolation.

9. Procedure

Thaw all reagents directly before starting the test, keep them cool (+2 °C to +8 °C), and use within 4 hours.

9.1 Procedure with RNA extraction

	Procedure with RNA extraction
1	Add 100 µl of **Solution B** to the lysis buffer per extraction run in a 96-well plate format.
2	Perform RNA extraction according to your laboratory's standard procedure.
3	Pipette 15 µL of **Solution A** into each PCR reaction tube/well of the microtiter plate.
4	Add 10 µL of **Positive Control** and 10 µL of **Negative Control** per run in respective wells. Add 10 µL of **eluate from RNA extraction** per remaining reaction tube/well of the microtiter plate.
5	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
6	Briefly centrifuge the microtiter plate or reaction tubes.
7	Place the filled plate/reaction tubes in the qPCR cycler.

9.1 Procedure for direct testing

	Procedure for direct testing (without RNA extraction)
1	*Optional: mix sample 1:1 with molecular grade water*
2	*Optional: heat sample at 95 °C for 5 minutes*
3	Prepare the reaction solution: add 100 µL of **Solution B** to the vial of **Solution A**; mix/shake briefly and centrifuge if necessary. Do not vortex!
4	Pipette 15 µL of the **reaction solution** into each PCR reaction tube/well of the microtiter plate.
5	Add 10 µL of **Positive Control** and 10 µL of **Negative Control** per run in respective wells. Add 10 µL of **solubilised patient sample** per remaining reaction tube/well of the microtiter plate.
6	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
7	Briefly centrifuge the microtiter plate or reaction tubes.
8	Place the filled plate/reaction tubes in the qPCR cycler.

10. Instrument Settings

The res4plex *direct* RT-PCR test was validated with the Roche LightCycler® 480 II and with the BioRad qPCR cycler CFX Opus 96™. In general, FRIZ *direct* PCR tests are compatible with any qPCR cycler (e.g., MIC Magnetic Induction Cycler, ABI QuantStudio 5, AJ qTOWER, Roche LightCycler® 96, Roche LightCycler® 480 I) but these and other cyclers may lead to altered Ct values and results. For basic information on programming your qPCR cycler, please refer to the user manual of the qPCR cycler used. The following thermal profile is to be used.

Steps	Temperature [°C]	Time	Number of cycles
Reverse transcription	55	10 min	1x
Initial denaturation	95	2 min	1x
Denaturation Amplification/Elongation	95 63	5 sec 15 sec	45x

Channel settings depend on qPCR cycler as well as the actual test used (see Appendix, p. 14 for detailed information on different test designs). Exemplary (Item # FBC107-4-1B) settings for a qPCR cycler are given below.

	SARS-CoV-2		Influenza A	RSV (A&B)	Influenza B	Internal Control
	N-gene	E-gene	MP-gene	N-gene	NS-gene	artificial RNA
Reporter dye	FAM	FAM	HEX	Red 610	Cy5	Cy5.5
Colour	green	green	yellow-green	orange	red	far-red
Emission [nm]	520	520	560	610	670	700
Quencher	Black Hole Quencher					

11. Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value.

- The Negative Control must not show amplification curves in all channels except for the IC channel.
- The Positive Control must show amplification curves in all channels. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- The IC for negative samples must show a positive amplification curve with a Ct value comparable to the IC Ct value in the Negative Control.
- The IC for positive samples should show a positive amplification curve with a Ct value comparable to the IC Ct value in the Negative Control.
- A significantly higher IC Ct value in samples compared to the IC Ct value in the Negative Control indicates procedural problems (e.g., purification problems or RT-PCR inhibition).
- A high concentration of detectable viral RNA in the sample can lead to reduced or absent IC signals. In such cases the result for the IC can be neglected.

The results support the differential diagnosis of infections with SRS-CoV-2, Influenza viruses and RSV. The viral RNA is generally detectable in respiratory samples during the acute phase of the infection. Positive results indicate the presence of the respective RNA, but do not exclude a co-infection with other pathogens. Co-infections of two or more viruses are possible, but with a very low probability of occurrence and therefore not included in the following table (exemplary interpretation of the results).

SARS-CoV-2 (FAM)	Influenza A (HEX)	RSV (Red 610)	Influenza B (Cy5)	Internal Control (Cy5.5)	Result	Interpretation
Positive	Negative	Negative	Negative	Positive/ Negative	Valid	SARS-CoV-2 detected
Negative	**Positive**	Negative	Negative	Positive/ Negative	Valid	Influenza A detected
Negative	Negative	**Positive**	Negative	Positive/ Negative	Valid	RSV detected
Negative	Negative	Negative	**Positive**	Positive/ Negative	Valid	Influenza B detected
Negative	Negative	Negative	Negative	**Positive**	Valid	No SARS-CoV-2, Influenza A, Influenza B or RSV detected
Negative	Negative	Negative	Negative	Negative	**Invalid!**	The test result cannot be evaluated.

A negative result does not exclude the presence of SARS-CoV-2, Influenza viruses or RSV, as results depend on correct sampling, the absence of inhibitors and sufficient RNA to be detected. Invalid results may be obtained if the sample contains inhibitors that prevent lysis, transcription and/or amplification or detection of the target nucleic acids. For information on known interfering substances, please refer to the section "Important Points before Starting/Sample Material".

12. Limitations of the Method

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.

The detection of analyte target does not mean that corresponding viruses 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.

Mutations or polymorphisms in primer and probe binding regions can interfere with the detection of new variants that may result in false negative results.

13. Analytical Performance

13.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of both, synthetic target-specific RNA and inactivated viruses spiked in a negative human naso-/oropharyngeal matrix. The analytes were tested in 24 replicates per concentration on a Bio-Rad CFX Opus 96. The 95% detection limit (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 9.3.1 software.

Analytical sensitivity	RNA (copies/reaction)	Virus in matrix ($TCID_{50}$/reaction)
SARS-CoV-2 LoD (95 CI)	8.17 (6.55 – 10.16)	8.75 (5.77 – 14.00) copies/reaction
Influenza A LoD (95 CI)	26.61 (24.31– 28.99)	0.469 (0.457– 0.481)
Influenza B LoD (95 CI)	13.82 (13.41 – 14.24)	0.0034 (0.0016 – 0.0062)
RSV A LoD (95 CI)	7.78 (7.24 – 8.32)	0.020 (0.014 – 0.027)
RSV B LoD (95 CI)	9.18 (6.84 – 12.22)	0.00037 (0.00016 – 0.00072)

13.2 Analytical Specificity

In silico analyses for potential cross-reactivities and inclusivity was performed with potentially co-sampled organisms using BLAST Alignment with the sequences stored in the NCBI database (see Appendix, p. 12). Identities of the primers and probes of less than 80% to the target sequence were considered not significant.

The *in silico* analyses showed an identity of more than 80% in few organisms: RSV B primers and probe showed a sequence identity of more than 80% to RSV A. Since the res4plex *direct* RT-PCR test does not differentiate between RSV A and RSV B, the sequence homologies can be neglected. All other sequence identities of more than 80% in the analysis showed that no amplification is possible due to the mapped distance of the primers to each other.

In addition to *in silico* analyses, the specificity of the test was determined *in vitro* by examining human pathogenic bacteria and viruses (see Appendix, p. 11). The measurement was performed in triplicates. None of the pathogens tested gave a positive signal in the res4plex *direct* RT-PCR test (all Internal Controls valid).

14. Interfering Substances

To investigate the effect of potentially interfering substances on the performance of the res4plex *direct* RT-PCR testing, the highest concentration of the substance potentially present in naso-/oropharyngeal samples was added to a negative clinical matrix with and without heat-inactivated SARS-CoV-2, Influenza A, Influenza B and RSV.

In addition, a control sample with matrix and heat-inactivated virus, but without potentially interfering substance was used as reference. Each condition was tested in triplicates. Potentially interfering substances, their active ingredient, the amount of medication applied as well as the res4plex *direct* RT-PCR test results are summarised in the Appendix, p. 13. Viral RNA of all targets was clearly identified in the presence of the tested potentially interfering substances at relevant concentrations (see Appendix, p. 13).

15. Diagnostic Performance

The diagnostic sensitivity of the res4plex *direct* RT-PCR test using RNA extraction is strongly dependent on the RNA extraction method used to isolate RNA from biological specimens. It is the responsibility of the user to qualify the extraction methods used for RNA isolation from biological samples.

For direct testing (without RNA extraction) the evaluation of the clinical performance was carried out with serial dilutions (3x, 30x and 300x LoD) of different isolates of SARS-CoV-2, Influenza A, Influenza B and RSV spiked in negative human naso-/oropharyngeal matrix. Negative samples were analysed in parallel with a CE-certified test kit from another manufacturer.

	SARS-CoV-2 positive samples	Influenza A positive samples	Influenza B positive samples	RSV positive samples	Negative samples
res4plex *direct* RT-PCR positive	108	108	102	105	0
res4plex *direct* RT-PCR negative	0	0	0	0	500
Sensitivity	**100%**	**100%**	**100%**	**100%**	**-**
Specificity	**100%**	**100%**	**100%**	**100%**	**-**

16. 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.

17. Symbols:

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

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18. Appendix

18.1 Microorganisms tested for cross-reactivity

Organism	Quantity per PCR	Result
Acinetobacter baumannii (gDNA)	1.0E+06 Genome copies	Negative
Bacillus cereus (gDNA)	1.0E+06 Genome copies	Negative
Corynebacterium bovis (gDNA)	1.0E+06 Genome copies	Negative
Enterococcus faecium (gDNA)	1.0E+06 Genome copies	Negative
Haemophilus influenzae (gDNA)	1.0E+06 Genome copies	Negative
Human coronavirus, NL63 (gRNA)	1.0E+06 Genome copies	Negative
Human coronavirus, 229E (gRNA)	1.0E+06 Genome copies	Negative
Human coronavirus, HKU1 (gRNA)	1.0E+06 Genome copies	Negative
Human coronavirus, OC43 (gRNA)	9.0E+04 Genome copies	Negative
Legionella pneumophila (gDNA)	8.0E+04 Genome copies	Negative
MERS Coronavirus (gRNA)	6.3E+04 Genome copies	Negative
Lactobacillus salivarius (gDNA)	1.0E+06 Genome copies	Negative
Mycobacterium tuberculosis (gDNA)	1.0E+06 Genome copies	Negative
Neisseria meningitidis (gDNA)	1.0E+06 Genome copies	Negative
Pseudomonas aeruginosa (gDNA)	1.0E+06 Genome copies	Negative
Staphylococcus aureus, Strain TCH70 (gDNA)	1.0E+06 Genome copies	Negative
Staphylococcus.epidermidis M23864:W2 (gDNA)	1.0E+06 Genome copies	Negative
Streptococcus mutans (gDNA)	1.0E+06 Genome copies	Negative
Parainfluenzavirus 1 (gRNA)	7.5E+04 Genome copies	Negative
Parainfluenzavirus 2 (gRNA)	6.5E+04 Genome copies	Negative
Parainfluenzavirus 3 (gRNA)	9.0E+04 Genome copies	Negative
Parainfluenzavirus 4 (gRNA)	7.0E+04 Genome copies	Negative
Rhinovirus (gRNA)	1.0E+05 Genome copies	Negative
Enterovirus D68 (gDNA)	1.0E+06 Genome copies	Negative

18.2 In silico analyses for cross reactivity and inclusivity of the used primers and probes: Identities of primers and probes of less than 80% to the target sequence were considered not significant (cns).

Organism (identity)	SARS-CoV-2	Flu A	Flu B	RSV A	RSV B	IC
Human coronavirus 229E	cns	cns	cns	cns	cns	cns
Human coronavirus OC43	cns	cns	cns	cns	cns	cns
Human coronavirus HKU1	cns	cns	cns	cns	cns	cns
Human coronavirus NL63	cns	cns	cns	cns	cns	cns
SARS-CoV	100%	cns	cns	cns	cns	cns
MERS-CoV	cns	cns	cns	cns	cns	cns
Adenovirus	cns	cns	cns	cns	cns	cns
Humanes metapneumovirus	cns	cns	cns	cns	cns	cns
HPIV1	cns	cns	cns	cns	cns	cns
HPIV2	cns	cns	cns	cns	cns	cns
HPIV3	cns	cns	cns	cns	cns	cns
HPIV4	cns	cns	cns	cns	cns	cns
Influenza A Virus	cns	100%	cns	cns	cns	cns
Influenza B Virus	cns	cns	100%	cns	cns	cns
Influenza C Virus	cns	cns	cns	cns	cns	cns
Enterovirus/Rhinovirus	cns	cns	cns	cns	cns	cns
RSV A	cns	cns	cns	100%	> 80%	cns
RSV B	cns	cns	cns	cns	100%	cns
Rubella Virus	cns	cns	cns	cns	cns	cns
Parechovirus	cns	cns	cns	cns	cns	cns
Chlamydia pneumoniae	cns	cns	cns	cns	cns	cns
Haemophilus influenzae	cns	cns	cns	cns	cns	cns
Legionella pneumophila	cns	cns	cns	cns	cns	cns
Mycobacterium bovis subsp. Bovis	cns	cns	cns	cns	cns	cns
Streptococcus pneumoniae	cns	cns	cns	cns	cns	cns
Streptococcus pyogenes	cns	cns	cns	cns	cns	cns
Bordetella pertussis	cns	cns	cns	cns	cns	cns
Mycoplasma pneumoniae	cns	cns	cns	cns	cns	cns
Pneumocystis jirovecii	cns	cns	cns	cns	cns	cns
Candida albicans	cns	cns	cns	cns	cns	cns
Corynebacterium diphtheriae	cns	cns	cns	cns	cns	cns
Bacillus anthracis	cns	cns	cns	cns	cns	cns
Moraxella catarrhalis	cns	cns	cns	cns	cns	cns
Neisseria elongata	cns	cns	cns	cns	cns	cns
Neisseria meningitidis	cns	cns	cns	cns	cns	cns
Pseudomonas aeruginosa	cns	cns	cns	cns	cns	cns
Staphylococcus aureus	cns	cns	cns	cns	cns	cns
Staphylococcus epidermidis	cns	cns	cns	cns	cns	cns
Streptococcus salivarius	cns	cns	cns	cns	cns	cns
Leptospiraceae	cns	cns	cns	cns	cns	cns
Chlamydia psittaci	cns	cns	cns	cns	cns	cns
Coxiella burnetii	cns	cns	cns	cns	cns	cns
Mycobacterium tuberculosis	cns	cns	cns	cns	cns	cns

18.3 Potentially interfering substances

Medication (trade name)	Active ingredient per unit	% (v/v)*	Result SARS-CoV-2	Result Influenza A	Result Influenza B	Result RSV	Result IC
ratioAllerg® Hay fever spray	50 µg Beclomethasone dipopionate	20	3/3	3/3	3/3	3/3	3/3
Otri-Allergie Hay fever spray	50 µg Fluticasone propionate	20	3/3	3/3	3/3	3/3	3/3
MometaHEXAL® Hay fever spray	50 µg Mometasone Fuorate	35	3/3	3/3	3/3	3/3	3/3
NASACORT® Nasal Spray	55 µg triamcinolone acetonide	30	3/3	3/3	3/3	3/3	3/3
Vividrin® akut Nasal Spray	140 µg Azelastine hydrochloride	30	3/3	3/3	3/3	3/3	3/3
Nasivin® Nasal Spray	22,5 µg Oxymetazoline hydrochloride	10	3/3	3/3	3/3	3/3	3/3
NASENSPRAY Heumann	90 µg Xylometazolinhydrochlorid	20	3/3	3/3	3/3	3/3	3/3
Syntaris® Nasal Spray	25 µg Flunisolide	20	3/3	3/3	3/3	3/3	3/3
Rhinocort Topinasal Nasal Spray	64 µg Budesonide	10	3/3	3/3	3/3	3/3	3/3
CromoHEXAL® Eye drops	ca 400 µg Sodium agglomoglicate	5	3/3	3/3	3/3	3/3	3/3
Tobrex® Eye drops	ca 60 µg Tobramycin	5	3/3	3/3	3/3	3/3	3/3
LISTERINE® Cool Mint Mouthwash	Ethanol (n.a.)	25**	3/3	3/3	3/3	3/3	3/3
Chlorhexamed® FLUID 0,1%	15 µg Chlorhexidine bis (D-gluconate)	50	3/3	3/3	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	3/3	3/3
Octenident® Mouth rinse solution	Octenidine (n.a.)	25**	3/3	3/3	3/3	3/3	3/3
Human blood	n.a.	5	3/3	3/3	3/3	3/3	3/3
Mucin	n.a.	5	3/3	3/3	3/3	3/3	3/3

* Concentration of medication in the medication-matrix mixture. It is assumed that the volume of one application is completely transferred into the sample.

** Higher concentrations of medication are prone to cause invalid or false negative results

18.4 Channel Settings

Possible combinations of the res4plex *direct* RT-PCR test and their respective channel settings are given below. For further information please contact the technical support of FRIZ Biochem.

Dye/Channel	**Cyan 500**	**FAM**	**HEX**	**Red 610**	**Cy5**	**Cy5.5**
Emission [nm]	480	520	560	610	670	700
Item #						
FBC 107-**4**-1A	Flu A	CoV2_N&E	Flu B	RSV	IC	
FBC 107-**4**-1B		CoV2_N&E	Flu A	RSV	Flu B	IC
FBC 107-**4**-2A	Flu A&B	CoV2_N	CoV2_E	RSV	IC	
FBC 107-**4**-2B		Flu A&B	CoV2_E	CoV2_N	RSV	IC
FBC 107-**4**-3A	Flu A	CoV2_N	CoV2_E	Flu B	IC	
FBC 107-**4**-3B		Flu A	CoV2_E	CoV2_N	Flu B	IC
FBC 107-**3**-1		Flu A	IC	Flu B	RSV	
FBC 107-**3**-2		Flu A	IC	CoV2_N&E	Flu B	
FBC 107-**3**-3		Flu A&B	IC	CoV2_N&E	RSV	
FBC 107-**3**-4		Flu A&B	IC	CoV2_N	CoV2_E	
FBC 107-**2**-1		CoV2_N&E	IC		Flu A&B	
FBC 107-**2**-2		CoV2_N	IC		CoV2_E	
FBC 107-**2**-3		Flu A	IC		Flu B	
FBC 107-**2**-4		Flu A&B	IC		RSV	
FBC 107-**1**-1		CoV2_N&E	IC			
FBC 107-**1**-2		Flu A&B	IC			
FBC 107-**1**-3		RSV	IC			
FBC 107-**1**-4		CoV2_N&E, Flu A&B	IC			
FBC 107-**1**-5		Flu A&B, RSV	IC			
FBC 107-**1**-6		CoV2_N&E, Flu A&B, RSV	IC			

CoV2_N = SARS-CoV-2 with primer, probes for its N-gene region
CoV2_E = SARS-CoV-2 with primer, probes for its E-gene region
CoV2_N&E = SARS-CoV-2 with primer, probes for its N-gene region as well as its E-gene region
Flu A = Influenza A with primer, probes for its MP-gene region
Flu B = Influenza B with primer, probes for its NS-gene region
Flu A&B = Influenza A and B with primer, probes for its MP-gene region as well as its NS-gene region
RSV = Respiratory Syncytial Virus A and B with primer, probes for their (common) N-gene region

A quick Start Protocol comes with each item summarising instructions for use as well as detailing channel attribution and result interpretation.