Instructions for Use

In-vitro-Diagnostic

IVD

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

Ref.No.:
FBC107-Ax
FBC107-Bx
FBC107-Cx

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

Content

1. Name of the Device

res4plex *direct* RT-PCR

2. Intended Purpose

The res4plex *direct* RT-PCR test is an assay for in vitro examination of viral RNA in nasal, nasopharyngeal or oral/oropharyngeal swabs to provide information to aid to diagnose symptomatic or asymptomatic patients under suspicion of respiratory diseases: SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus A/B (RSV A/B).

The IVD medical device detects the RNA of the aforementioned pathogens by qualitative measurements based on RT-qPCR and is intended for use in medical laboratories or health institutions by laboratory personnel specifically trained in RT-qPCR and *in vitro* diagnostic techniques. It has to be used in combination with conventional nucleic acid extraction systems for RNA extraction and RT-qPCR cyclers for detection and analysis.

3. Pathogen Information

Influenza viruses are single-stranded and belong to the family of Orthomyxoviridae [1]. The genetic material of membrane-enveloped viruses consists of eight different RNA segments. The genome codes for 10 to 14 known proteins, including the surface proteins hemagglutinin (HA) and neuraminidase (NA) (Figure 1) [2, 3]. There are three different types of influenza viruses known: A, B and C [4]. Influenza C is rarely reported as a cause of human illness, probably because most cases are subclinical. Influenza C has not been associated with epidemic disease yet [5]. The other two influenza viruses, A and B, are more important regarding the possibility of infecting human subjects. Influenza A can cause both seasonal and pandemic influenza, whereas influenza B is responsible for seasonal influenza only [1]. Influenza A viruses are further classified into subtypes according to the combination of HA and NA [6]. There are 18 different H subtypes and 11 different N subtypes. Eight H subtypes (H1, H2, H3, H5, H6, H7, H9, H10) and six N subtypes (N1, N2, N6, N7, N8, and N9) have been detected in humans [5]. Influenza B viruses are not classified into subtypes but can be subclassified into two lineages. Currently, circulating influenza type B viruses belong to either the B/Yamagata or B/Victoria lineages [6].

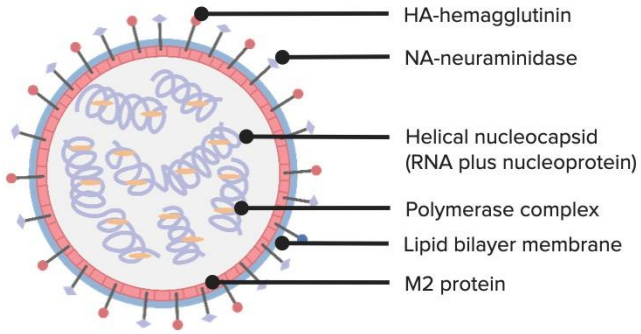

Figure 1: General structure of Influenza virus [7].

Influenza infections are common worldwide. Influenza epidemics usually occur in the winter half-year, peaks for an average of 8 to 10 weeks but can last much longer in individual years [4]. Transmission of influenza viruses occurs from infectious people to susceptible people through large virus-containing droplets and aerosols produced by coughing, sneezing or talking [3]. The virus can also be potentially spread by the hands or fomites contaminated with the influenza virus with subsequent inoculation into the upper respiratory tract and by an aerosol transmission during aerosol-generating procedures [6].

Symptoms typical of influenza are characterized by sudden illness, fever, cough or sore throat, and muscle aches and/or headache. Other symptoms may include general weakness, sweating, rhinorrhea, and rarely nausea/vomiting and diarrhea [8].

SARS-CoV-2 is classified within the genus *Betacoronavirus* (subgenus *Sarbecovirus*) of the family *Coronaviridae* [9]. Betacoronaviruses also include SARS-CoV, MERS-CoV (Middle East respiratory syndrome coronavirus), and the human coronaviruses (HCoV) HKU1 and OC43 that circulate as cold viruses [10]. Infections with SARS-CoV-2 lead to a new disease profile called COVID-19 (Corona Virus Disease-2019) [11]. The first human cases of COVID-19 were reported from Wuhan City, China, in December 2019 [12].

SARS-CoV-2 is an enveloped, positive sense, single-stranded ribonucleic acid (RNA) virus with a 30-kb genome. The genome encodes for non-structural proteins (essential in forming the replicase transcriptase complex), four structural proteins (spike (S), envelope (E), membrane (M), nucleocapsid (N)) and putative accessory proteins [9]. The S-, E- and M- proteins are incorporated into the viral membrane that envelops the nucleocapsid composed of N-protein (nucleoprotein) and viral genome (Figure 2). The S-protein is responsible for entry into the host cell [13].

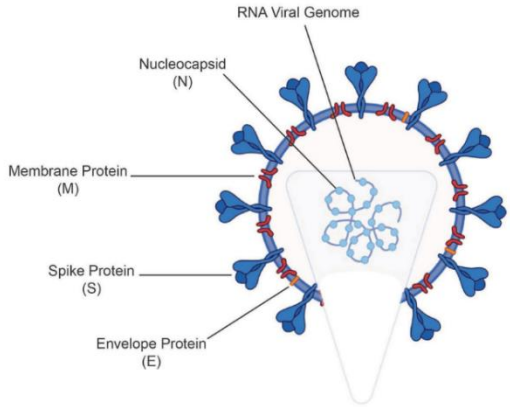

Figure 2: General structure of SARS-CoV-2 [14].

SARS-CoV-2 variants are classified as VOI (variant of interest) or VOC (variant of concern). A virus variant is classified as VOI if the variant has a phenotype change or carries mutations that probably or definitely affect the phenotype and has caused several case clusters or cases in different countries. A VOC is a virus variant with altered pathogen characteristics that have been shown to adversely affect epidemiology (especially increased transmissibility), clinical presentation (especially increased virulence), or the effectiveness of countermeasures, diagnostic detection methods, vaccines, or therapeutics, respectively. In the following Table 1 the current known VOCs of SARS-CoV-2 are listed [15]. Additional VOI are known. The target sequences of the res4plex *direct* RT-PCR test covers all VOC in Table 1. The transmission of SARS-CoV-2 usually occurs through infected persons via droplet infection and aerosols, whereby close contact favors transmission [16]. Indirect contact transmission involving contact of a susceptible host with a contaminated object or surface (fomite transmission) may also be possible [17].

Table 1: Variants of concern (VOC) of SARS-CoV-2 as per October 2023

WHO	PANGO NOMENCLATURE	FIRST DETECTION
Alpha	B.1.1.7	Great Britain (September 2020)
Beta	B.1.351	South Africa (May 2020)
Gamma	P.1 alias B.1.1.28.1	Brazil (November 2020)
Delta	B.1.617.2	India (October 2020)
Omikron	B.1.1.529	Botswana (November 2021)

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 [18].

Respiratory Syncytial Virus (RSV), discovered in 1956, is one of the most common causes of childhood illness [19]. It is a single-stranded (ss), negatively oriented (-), unsegmented RNA virus of the family Pneumoviridae (genus Orthopneumovirus) (see Figure 3). It has a bilayer lipid envelope in which glycoproteins are incorporated, including a fusion (F) and an adhesion (G) protein. Two subtypes**,** A and B, exist which differ in the antigenic structure of the G protein [20]. These subtypes comprise several genotypes [21].

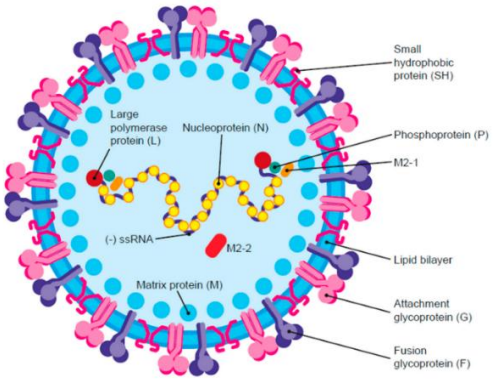

Figure 3: Respiratory syncytial virus genomic overall structure of genes coding for proteins [22].

RSV causes yearly winter epidemics and shows in many countries a 2-year rhythmicity, in which an early-onset strong season is followed by a later-onset weaker season [23]. However, in 2020 in Europe, the RSV winter season was nearly noticeable due to the protective measures in the context of the COVID-19 pandemic, whereas in the following year a strong, early onset RSV season occurred [24]. The transmission of RSV occurs primarily by droplet infection from an infectious person to a contact person. Transmission is also thought to be possible indirectly through contaminated hands, objects and surfaces [20]. People infected with RSV are usually contagious for 3 to 8 days. However, some infants, and people with weakened immune systems, can continue to spread the virus even after they stop showing symptoms for as long as four weeks [25]. Adolescents and adults play a role as asymptomatic or low-symptomatic carriers [20].

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, but infection 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) [26].

4. Testing Principle

The res4plex *direct* RT-PCR test is a multiplex RT-qPCR test for the detection and differentiation of SARS-CoV-2, influenza A, influenza B and RSV (A&B).

A separate full process run control (Internal Control; IC) is added to each sample prior to RNA extraction and serves as control for nucleic acid isolation from the biological specimen as well as for RT-qPCR.

The RNA eluate is added to the ready-to-use reaction solution that contains all reagents necessary for RT-qPCR. RT-qPCR analysis can be performed on various RT-qPCR cyclers.

The res4plex *direct* RT-PCR test contains primer and probes specific for the targets listed in Table 2 and the 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 intact probes.

Table 2: Target genes of res4plex direct RT-PCR test

ANALYTE	GENE(S)
SARS-CoV-2	N gene (nucleotides 14 to 83) and E gene (nucleotides 25 to 137)
Influenza A	MP gene (nucleotides -1 to 161)
Influenza B	NS gene (nucleotides 688 to 818)
RSV A/B	N gene (nucleotides 999 to 1134)

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.

5. Package Content

Each kit contains the following vials which are sufficient for 96 reactions (see Table 3).

Table 3: Package content of res4plex direct RT-PCR

MATERIAL	LID COLOUR	#VIALS; VOLUME	#RXNS.	COMMENT
Solution A	green	1x; 1050 µL	96	Reaction mix (buffer, enzymes, primer and probes)
Internal Control	blue	1x; 400 µL	96	Internal Control (artificial nucleic acid target)
Positive Control*	red	1x; 50 µL	4	Nucleic acids of SARS-CoV-2, influenza A, influenza B and RSV

*One Positive Control/kit is included. If more Positive Control is needed, it can be purchased separately: Reference number FBC107-PC.

6. Configurations

The res4plex *direct* RT-PCR kit is available in the following variants:

Table 4: Configurations of res4plex direct RT-PCR

REFERENCE NUMBER	CONFIGURATION	TARGETS
FBC107-Ax	suitable for use on a qPCR instrument with at least 5 detection channels (Cyan500/FAM/HEX/Red610/Cy5)	Flu A; Flu B; SARS-CoV-2; RSV (A/B)
FBC107-Bx	suitable for use on a qPCR instrument with at least 5 detection channels (FAM/HEX/Red610/Cy5/Cy5.5)	Flu A; Flu B; SARS-CoV-2; RSV (A/B)
FBC107-Cx	suitable for use on a qPCR instrument with at least 4 detection channels (FAM/HEX/Red610/Cy5)	Flu A/B; SARS-CoV-2; RSV (A/B)

7. Additional Equipment and Reagents (not provided)

- qPCR cycler (channel requirements see Table 4)
- Disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- sterile filter-tips for PCR testing (DNA/RNA- and RNase-free)
- Table centrifuge
- RNA isolation kit (IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 by PerkinElmer chemagen Technologie GmbH or similar device)
- Negative Control (no-template control, molecular grade water, or any other negative control according to the laboratory´s standard procedure)

8. 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 8 hours. Exposure to light should be avoided. You may thaw and refreeze Solution A and Internal Control up to 4 times. The package bears an expiry date, after which no quality guarantee can be given.

9. Warnings, Safety Precautions and Additional Information Access

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. If the user makes changes to the product or the application instructions, results may not correlate with the intended purpose.

All serious incidents relating to the kit must be notified to the manufacturer and the national competent authority of the EU Member State where the laboratory and/or patient is located.

- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to invalid 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.
- 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.
- 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 after performing qPCR reaction.
- If contamination of the qPCR cycler is suspected, cleaning and maintenance must be carried out according to the system´s manual.
- Safety Data Sheets (SDS) are available for download via https://frizbiochem.de/downloads/
- Pending EUDAMED entry, the Safety and Performance Summary (SSP) can be downloaded via www.frizbiochem.de

10. Specimen Collection, Handling, Transport, and Storage

Proper sample collection, storage and transport are critical for RT-PCR analyses in general. Inadequate sample collection, improper sample handling and/or transport can lead to false negative results. Therefore, ensure that:

- sample collection must be carried out by qualified healthcare professionals in accordance with applicable national laws and regulatory requirements.
- the instructions of the medical laboratory and/or the manufacturer of the sample collection system for sample collection, transport and storage are followed.
- the sample collector is instructed to collect the sample in accordance with applicable national laws, regulatory requirements and the instructions for use of the sampling tool.

Figure 4 shows an overview of the respective locations of the sampling points in the mouth/nose area. To avoid contamination of the sample, do not touch anything other than the sampling area with the swab tip.

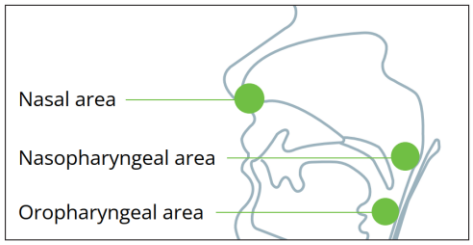

Figure 4: Sampling sites in the oral/oropharyngeal cavity

11. Important Points before Starting

This test is intended for use with samples derived from nasal, nasopharyngeal or oral/oropharyngeal. Other specimen types may cause invalid results.

In every RT-qPCR run one Positive Control and one Negative Control should be included. The Positive Control consists of nucleic acids of SARS-CoV-2, influenza A, influenza B and RSV. The Negative Control (e.g. molecular grade water) must be provided by the user. A failed Positive or Negative Control will invalidate the RT-qPCR run and the results must not be reported.

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. Performance evaluation studies have been conducted

using the IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 on a chemagic™ 360 instrument (PerkinElmer chemagen Technologie GmbH) for RNA isolation.

12. Test Procedure

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

Table 5: Test procedure of res4plex direct RT-PCR

TEST PROCEDURE	
	Sample preparation
1	Thaw all reagents completely.
2	Add Internal Control to the RNA preparation process in accordance to the laboratory´s standard procedure (e.g., add 4µL/sample to lysis buffer).
3	Perform RNA preparation according to your laboratory's standard procedure.
	RT-qPCR
4	Pipette 10 µL/well of Solution A into the PCR microtiter plate/reaction tubes.
5	Add 10 µL/well of eluate from RNA preparation; add 10 µL Positive Control per run; add 10 µL Negative Control per run.
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.
9	Start program.

13. Instrument Settings

The res4plex *direct* RT-PCR was validated with the Roche LightCycler® 480 II and BioRad CFX Opus 96™. In general, FRIZ Lab *direct* PCR tests are compatible with many RT-qPCR cyclers. The following thermal profile is to be used (see Table 6). An alternative thermal profile can be found in the appendix (see Table 12). Channel settings depend on the RT-qPCR cycler used and should be checked before starting the analysis (see Table 7 and Table 8). FBC107-Ax is suitable for RT-qPCR cyclers with at least the five channels Cyan 500, FAM, HEX, Red 610, and Cy5 (e.g., LightCycler® 480 II), FBC107-Bx is suitable for RT-qPCR cyclers with at least the five channels FAM, HEX, Red 610, Cy5, and Cy5.5 (e.g., BioRad CFX Opus 96™ or analytik jena qTOWERiris), and FBC107-Cx is suitable for RT-qPCR cyclers with at least the four channels FAM, HEX, Red 610, and Cy5 (e.g. MIC qPCR Cycler or LightCycler® 96). Make sure to activate the detection mode of your qPCR cycler before starting the analysis.

Table 6: Instrument Settings of res4plex direct RT-PCR

STEPS	TEMPERATURE [°C]	TIME	ACQUISITION	#CYCLES
Initial step	55	5 min	-	1
Denaturation	95	3 min	-	1
Amplification	95	5 sec	-	45
	60	15 sec	on	
	72	15 sec	-	

Table 7: Channel settings of res4plex direct RT-PCR (Ref. No.: FBC107-Ax and FBC107-Bx)

	SARS-CoV-2		Influenza B	RSV (A&B)	Influenza A	Internal Control (IC)	
	N-gene	E-gene	NS-gene	N-gene	MP-gene	artificial NA	
						FBC107-Ax	FBC107-Bx
Reporter dye	FAM	FAM	HEX	Red 610	Cy5	CY500	Cy5.5
Colour	green	green	yellow-green	orange	red	cyan	far-red
Emission	520	520	560	610	670	480	700
Quencher	Black Hole Quencher						

Table 8: Channel settings of res4plex direct RT-PCR (Ref.No.: FBC107-Cx)

	SARS-CoV-2		RSV (A&B)	Influenza A/B	Internal Control (IC)
	N-gene	E-gene	N-gene	MP/NS-gene	artificial NA
					FBC107-Cx
Reporter	FAM		Red 610	Cy5	HEX
Colour	green	green	orange	red	yellow-green
Emission	520	520	610	670	560
Quencher	Black Hole Quencher				

14. Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value (see Figure 5). For distribution of results within the channels see Table 9 and Table 10.

A positive signal is characterized by a sigmoidal curve exhibiting both a baseline phase and an exponential phase. Signals that do not exhibit this curve pattern, despite having a Ct value, are considered negative. The threshold shall be set within the exponential phase. It should be noted that in the case of low positive samples, the plateau phase might not be visible due to the cut off after 45 cycles. Thus, the presence of a plateau phase is not an essential requirement for defining a positive signal in such cases.

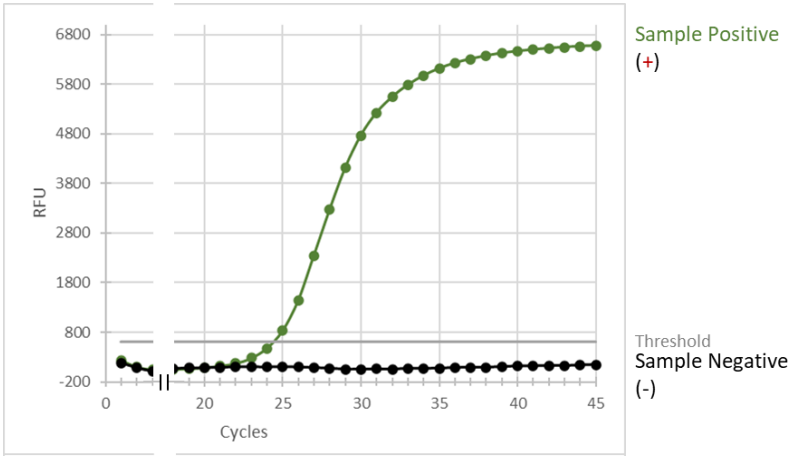

Figure 5: Exemplary amplification curves

- 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 should show a positive amplification curve in both positive and negative samples.

Table 9: Results of res4plex direct RT-PCR (Ref. No.: FBC107-Ax and FBC107-Bx)

					FBC107-Ax		FBC107-Bx	
SARS-CoV-2		**Influenza B**	**RSV (A&B)**	**Influenza A**	**Internal Control (IC)**			
N-gene	E-gene	NS-gene	N-gene	MP-gene	artificial NA			
FAM		HEX	Red 610	Cy5	CY500	Cy5.5	**Result**	**Interpretation**
+		–	–	–	+		Valid	SARS-CoV-2 detected.
–		+	–	–	+		Valid	Influenza B detected.
–		–	–	–	+		Valid	RSV detected.
–		–	–	+	+		Valid	Influenza A detected.
–		–	–	–	+		Valid	No SARS-CoV-2, Influenza B, RSV or Influenza A detected.
+/–		+/–	+/–	+/–	–		Invalid	The test result can not be evaluated.

Table 10: Results of res4plex direct RT-PCR (Ref. No.: FBC107-Cx)

				FBC107-Cx			
SARS-CoV-2		**RSV (A&B)**	**Influenza A/B**	**Internal Control (IC)**			
N-gene	E-gene	N-gene	MP/NS-gene	artificial NA			
FAM		Red 610	Cy5	HEX	**Result**	**Interpretation**	
+		–	–	+	Valid	SARS-CoV-2 detected.	
–		+	–	+	Valid	RSV detected.	
–		–	+	+	Valid	Influenza A/B detected.	
–		–	–	+	Valid	No SARS-CoV-2, Influenza B, RSV or Influenza A detected.	
+/–		+/–	+/–	–	Invalid	The test result can not be evaluated.	

15. Limitations of the Method

The results support the differential diagnosis of infections with SARS-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 pathogen, but do not exclude a co-infection with other pathogens.

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, extraction, transcription and/or amplification or detection of the target nucleic acids. For information on tested interfering substances, please refer to the Appendix.

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 they are the causative agents of clinical symptoms.

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

Human blood is a PCR inhibitor [27] and was found to interfere with res4plex *direct* RT-PCR at concentrations greater than 1% (v/v). Visible blood on the swab may impact RNA isolation and RT-qPCR and a false-negative result could occur.

The detection of a target analyte does not exclude the possibility of a co-infection with another target analyte since competitive interference may occur (see Competitive Interference in the appendix).

16.Analytical Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of synthetic target-specific RNA. The analytes were tested in 24 replicates per concentration on a Bio-Rad CFX Opus 96™. The 95% confidence interval (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 9.3.1 software (see Table 11).

Table 11: Limit of Detection

ANALYTE	LIMIT OF DETECTION (95 CI)
SARS-CoV-2	2.84 (2.18 – 3.53) copies/reaction
Influenza A H1N1 pdm09	5.26 (3.42 – 7.51) copies/reaction
Influenza A H3N2	5.30 (3.85 – 6.87) copies/reaction
Influenza B	3.65 (2.88 – 4.52) copies/reaction
RSV A	3.54 (1.65 – 6.54) copies/reaction
RSV B	3.17 (2.45 – 3.94) copies/reaction

16.2 Other Analytical Performance Parameters

Other analytical performance data, such as analytical specificity including cross-reactivity, endogenous and exogenous interfering substances, inclusivity and competitive interference as well as precision (repeatability and reproducibility) are shown in the Appendix.

17. Diagnostic Performance

In total 891 samples were analysed in an independent medical laboratory in August 2022. All samples were extracted using the IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 on a chemagic™ 360 instrument (PerkinElmer chemagen Technologie GmbH, 30 min protocol). Subsequently, samples were analysed in parallel with the res4plex *direct* RT-PCR test and a CE certified test from another manufacturer in direct comparison on a LightCycler® 480 II (Roche).

From the tested samples, 105 were positive for SARS-CoV-2, 77 were positive for influenza A, 54 were positive for influenza B and 83 were positive for RSV. Diagnostic sensitivity and specificity are summarized in the following:

SARS-CoV-2 samples

		positive	negative	
res4plex	positive	104	1	Sensitivity: 99.1% (94.8 - 100.0)
	negative	1	744	Specificity: 99.9% (99.3 - 100.0)

Influenza A samples

		positive	negative	
res4plex	positive	73	1	Sensitivity: 94.8% (87.2 - 98.6)
	negative	4	680	Specificity: 99.9% (99.2 - 100.0)

Influenza B samples

		positive	negative	
res4plex	positive	54	0	Sensitivity: 100.0% (93.4 - 100.0)
	negative	0	725	Specificity: 100.0% (99.5 - 100.0)

RSV samples

		positive	negative	
res4plex	positive	83	1	Sensitivity: 100.0% (95.7 - 100.0)
	negative	0	667	Specificity: 99.9% (99.2 - 100.0)

The diagnostic sensitivity of the res4plex *direct* RT-PCR test using RNA extraction is 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.

CE 0123 This product complies with the requirements of the Regulation (EU) *2017*/746 (*IVDR*) for in vitro diagnostic medical devices.

18. Symbols

Please note following symbol descriptions according to EN ISO 15223-1.

Graphic	Title	Description
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Manufacturer	Indicate the medical device manufacturer.
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.
	Use-by date	Indicates the date after which the medical device is not to be used.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.
	CE marking European Conformity	
	Catalogue number (Reference number)	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Content sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.
	Do not reuse	Indicates a medical device that is intended for one single use only.
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Keep away from sunlight	Indicates a medical device that needs protection from light sources.
	Unique device identifier	Indicates a carrier that contains unique device identifier information.

Document Version:

FBC107_IFU_EN	
Version 1.0 Date: 19.05.2022	First publication 19.05.2022
Version 2.0 Date: 16.11.2022	Revised Version Adjustment of Test Configurations
Version 3.0 Date: 08.03.2022	Revised Version Adjustment of Test Configurations
Version 4.0 Date: 08.03.2022	Revised Version Adjustment of Test Configuration; Correction of tipping errors
Version 5.0 Date: 27.10.2023	Revised Version Insertion of chapter 10: Specimen Collection, Handling, Transport, and Storage Adjustment of Instrument Settings (chapter 13): Denaturation and Amplification/Elongation times
Version 5.1 Date: 28.02.2024	Revised Version • Insertion of “symptomatic or asymptomatic” in Intended Purpose • Addition of gene regions in table 2 • Correction of volumes and #rxns + note for separate Positive Control in table 3 • Specification of additional equipment in chapter 7 • Correction of Transport, Storage and Stability (chapter 8) • Correction of Specimen Collection, Handling, Transport, and Storage (chapter 10) • More precise description of instrument settings (chapter 13) • Result interpretation changed to signal of IC needs to be positive (chapter 14) • Note added in chapter 15 that “the detection of a target analyte does not exclude the possibility of a co-infection with another target analyte since competitive interference may occur (see Competitive Interference in the appendix).” • Symbols updated (chapter 18) • Precision and Carryover/Cross-contamination data corrected (chapter 19.5 and 19.6)
Version 5.2 Date: 08.07.2024	Revised Version • Title of Symbol corrected (“Use-by date”) • Description of blood in specimen in chapter 15 changed
Version 5.3 Date: 17.09.2024	Revised Version • Reference 27 added to chapter 15 • Note highlighted in chapter 10 that the user requires his sample collectors to take appropriate samples in accordance with applicable national laws, regulatory requirements and the instructions for use of the sampling tool • Chapters 10.1/10.2 and 10.3 were omitted
Version 5.4 Date: 26.02.2025	Revised Version • Instrument settings alternative 2 added to chapter 13 • Gene region of influenza A changed in chapter 4 according to change in product version 2 • Update of inclusivity data in the Appendix chapter 19.3 according to change in product version 2

Version 5.5 Date: 15.07.2025	Revised Version • Alternative thermal profile moved from chapter 13 to the appendix • Deletion of “List of Tables” • Minor editorial changes

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19. Appendix

19.1 Alternative Thermal Profile

Table 12: Alternative Instrument Settings of res4plex direct RT-PCR

STEPS	TEMPERATURE [°C]	TIME	ACQUISITION	#CYCLES
Initial step	55	10 min	-	1
Denaturation	95	2 min	-	1
Amplification	95	10 sec	-	45
	60	30 sec	on	

19.2 Cross-reactivity

Cross-reactivity was tested with in silico analysis as well as with wet-lab testing.

For the *in silico* analysis, all primer and probe sequences of the res4plex *direct* RT-PCR test were aligned to the organisms in Table 13 using nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 13: In silico analysis for cross-reactivity of the used primers and probes. Identities of primers and probes of less than 80% to the target sequence were considered no significant (-).

Organism	SARS	Flu A	Flu B	RSV A	RSV B	IC
Human coronavirus 229E	-	-	-	-	-	-
Human coronavirus OC43	-	-	-	-	-	-
Human coronavirus HKU1	-	-	-	-	-	-
Human coronavirus NL63	-	-	-	-	-	-
SARS-CoV*	100%	-	81% to fw primer	-	-	-
MERS-CoV	-	-	-	-	-	-
Adenovirus	-	-	-	-	-	-
Humanes metapneumovirus	-	-	-	-	-	-
HPIV1	-	-	-	-	-	-
HPIV2	-	-	-	-	-	-
HPIV3	-	-	-	-	-	-
HPIV4	-	-	-	-	-	-
Influenza A Virus	-	100%	-	-	-	-
Influenza B Virus	-	-	100%	-	-	-
Influenza C Virus	-	-	-	-	-	-
Enterovirus/Rhinovirus	-	-	-	-	-	-
RSV A	-	-	-	100%	> 80%	-
RSV B	-	-	-	> 80%	100%	-
Rubella Virus	-	-	-	-	-	-
Parechovirus	-	-	-	-	-	-
Epstein Barr virus	-	-	-	-	-	-
Human cytomegalovirus	-	-	81% to fw primer	-	-	-
Measles virus	-	-	-	-	-	-
Mumps virus	-	-	-	-	-	-
Norovirus	-	-	-	-	-	-
Rotavirus	-	-	-	-	-	-
Chlamydia pneumoniae	-	-	-	-	-	86% to rev primer
Haemophilus influenzae	83% to E-gene primer	-	-	-	-	-

Organism	SARS	Flu A	Flu B	RSV A	RSV B	IC
Legionella pneumophila	-	-	-	-	-	-
Mycobacterium bovis subsp. *bovis*	-	-	-	-	-	-
Streptococcus pneumoniae	-	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-	-
Bordetella pertussis	-	-	-	-	-	-
Mycoplasma pneumoniae	-	-	-	-	-	-
Pneumocystis jirovecii	-	-	-	-	-	-
Corynebacterium diphtheriae	-	90% to probe	-	-	-	-
Bacillus anthracis	-	-	-	-	-	-
Moraxella catarrhalis	-	-	-	-	-	-
Neisseria elongata	-	-	-	-	-	-
Neisseria meningitidis	86% to N-gene primer	-	-	-	-	-
Pseudomonas aeruginosa	89% to E-gene primer	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-
Streptococcus salivarius	-	-	-	-	-	-
Leptospiraceae spp.	-	-	-	-	-	-
Chlamydia psittaci	-	-	-	-	-	-
Coxiella burnetii	-	-	-	-	-	-
Mycobacterium tuberculosis	-	-	-	-	-	-
Escherichia coli	83% to E-gene primer	-	81% to fw primer	-	-	-
Klebsiella pneumoniae	-	-	85% to rev primer	-	-	-
Lactobacillus spp.	-	-	-	-	-	-
Candida albicans	-	-	90% to rev primer	-	-	-
Aspergillus fumigatus	-	-	86% to fw primer	83% to rev primer	-	-
Candida glabrata	-	-	81% to fw primer	-	-	81% to rev primer
Cryptococcus neoformans	82% to E-gene primer	-	90% to fw primer	-	-	-

*Due to the homology of the E gene, cross-reactivity of SARS-CoV-1 is expected.

The analytical specificity of the res4plex *direct* RT-PCR test was further evaluated by testing a panel of 28 cultures consisting of 15 viral, 9 bacterial and 4 fungi strains representing common respiratory pathogens and those that could potentially cross-react with oligonucleotides from the test as indicated by the *in silico* analysis. Six replicates of each strain with highest possible concentrations were tested as spiked (10x LoD of each target) and unspiked group (3 replicates each).

In the spiked group, positive signals were observed for all four targets in each set of triplicate samples. In contrast, unspiked samples displayed positive signals only for the Internal Control, while the targets showed negative signals (Table 14-Table 16). In conclusion, the res4plex *direct* RT-PCR test demonstrates reliable analytical specificity with respect to cross-reactivity.

Table 14: Cross-reactivity analysis with viral templates

Nr.	Sample Type*	Organisms	Final Concentration	Measurement Unit	Spiked Sample		Unspiked Sample	
					Targets	Internal Control	Targets	Internal Control
1	Viral CF	Adenovirus 1	3.72E+07	TCID50/ml	detected (D)	detected (D)	not detected (ND)	detected (D)
2	Viral CF	Adenovirus 7	1.26E+05	TCID50/ml	D	D	ND	D
3	Viral CF	Enterovirus D	5.01E+04	TCID50/ml	D	D	ND	D
4	Viral CF	Human coronavirus 229E	1.29E+04	TCID50/ml	D	D	ND	D
5	RNA	Human coronavirus HKU1	1.00E+06	genome copies/ml	D	D	ND	D
6	Viral CF	Human coronavirus OC43	1.70E+04	TCID50/ml	D	D	ND	D
7	Viral CF	Human coronavirus NL63	3.55E+04	TCID50/ml	D	D	ND	D
8	Viral CF	Human cytomegalovirus	5.62E+03	TCID50/ml	D	D	ND	D
9	Viral CF	Human metapneumovirus	1.41E+04	TCID50/ml	D	D	ND	D
10	RNA	MERS coronavirus	1.00E+05	genome copies/ml	D	D	ND	D
11	Viral CF	Parainfluenza virus 1	1.38E+06	TCID50/ml	D	D	ND	D
12	Viral CF	Parainfluenza virus 2	1.41E+04	TCID50/ml	D	D	ND	D
13	Viral CF	Parainfluenza virus 3	3.39E+06	TCID50/ml	D	D	ND	D
14	Viral CF	Rhinovirus A	1.41E+04	TCID50/ml	D	D	ND	D
15	RNA	SARS-CoV-1	1.00E+05	genome copies/ml	D	D	3x SARS signal detected	D

*Viral CF = heat-inactivated viral culture fluid

Table 15: Cross-reactivity analysis with bacterial templates

Nr.	Sample Type	Organisms	Final Concentration	Measurement Unit	Spiked Sample		Unspiked Sample	
					Targets	Internal Control	Targets	Internal Control
16	DNA	*Chlamydia pneumoniae*	1.00E+06	genome copies/ml	detected (D)	detected (D)	not detected (ND)	detected (D)
17	DNA	*Escherichia coli*	1.00E+06	genome copies/ml	D	D	ND	D
18	DNA	*Haemophilus influenzae*	1.00E+06	genome copies/ml	D	D	ND	D
19	DNA	*Klebsiella pneumoniae*	1.00E+06	genome copies/ml	D	D	ND	D
20	DNA	*Neisseria meningitidis*	1.00E+06	genome copies/ml	D	D	ND	D

Nr.	Sample Type	Organisms	Final Concentration	Measurement Unit	Spiked Sample		Unspiked Sample	
					Targets	Internal Control	Targets	Internal Control
21	DNA	*Pseudomonas aeruginosa*	1.00E+06	genome copies/ml	D	D	ND	D
26	DNA	*Corynebacterium diphtheriae* subsp. *Diphtheriae*	1.00E+06	genome copies/ml	D	D	ND	D
27	DNA	*Legionella pneumophila*	1.00E+06	genome copies/ml	D	D	ND	D
28	DNA	*Streptococcus pyogenes*	1.00E+06	genome copies/ml	D	D	ND	D

Table 16: Cross-reactivity analysis with fungal templates

Nr.	Sample Type	Organisms	Final Concentration	Measurement Unit	Spiked Sample		Unspiked Sample	
					Targets	Internal Control	Targets	Internal Control
22	DNA	*Aspergillus fumigatus*	1.32E+06	genome copies/ml	detected (D)	detected (D)	not detected (ND)	detected (D)
23	DNA	*Candida albicans*	4.45E+06	genome copies/ml	D	D	ND	D
24	DNA	*Candida glabrata*	1.31E+06	genome copies/ml	D	D	ND	D
25	DNA	*Cryptococcus neoformans*	3.99E+06	genome copies/ml	D	D	ND	D

19.3 Endogenous and Exogenous Interfering Substances

Interfering substances (see Table 17) were spiked into artificial nasopharyngeal matrix at highest possible concentrations in 5 replicates. Samples were extracted using the IVD-1033-S chemagic™ Viral DNA/RNA 300 Kit H96 on a chemagic™ 360 instrument (PerkinElmer chemagen Technologie GmbH). Eluates were either tested directly (unspiked sample group) or after spiking with all target RNAs (SARS-CoV-2, influenza A, influenza B, RSV A, RSV B) at a concentration of 3x LoD (spiked sample group).

At indicated concentrations, except from human blood none of the tested substances showed interference with the res4plex *direct* RT-PCR test (see Table 17). Since amplification of influenza A was inhibited in presence of human blood at a concentration of 2% (v/v), lower concentrations of human blood (1%, 0.5% and 0.2%) were tested (see Table 18). At human blood concentrations of 1% (v/v) or lower, all target signals were detected.

Table 17: Tested potentially interfering substances

Category	Substance (trade name)	Concentration	Spiked Sample		Unspiked Sample	
			Targets	Internal Control	Targets	Internal Control
endogenous substances	Human genomic DNA	1 ng/µl	detected (D)	detected (D)	not detected (ND)	detected (D)
	Mucin	1 mg/ml	D	D	ND	D
	Human Blood	0.2 - 2% (v/v)	See Table 18	D	ND	D
anti-allergy medicine	Histamine dihydrochloride	1 mg/ml	D	D	ND	D
	DHU Histaminum Hydrochloricum D4	10 globules/ml	D	D	ND	D
anti-viral medicine	Arbidol	1 mg/ml	D	D	ND	D
	Amantadine	1 mg/ml	D	D	ND	D
	Lopinavir	1 mg/ml	D	D	ND	D
	Nirmatrelvir (Paxlovid®)	1.5 mg/ml	D	D	ND	D

Category	Substance (trade name)	Concentration	Spiked Sample		Unspiked Sample	
			Targets	Internal Control	Targets	Internal Control
	Oseltamivir	1 mg/ml	D	D	ND	D
	Peramivir	0.1 mg/ml	D	D	ND	D
	Remdesivir	0.5 mg/ml	D	D	ND	D
	Ribavirin	1 mg/ml	D	D	ND	D
	Ritonavir	1 mg/ml	D	D	ND	D
	Zanamivir	5 mg/ml	D	D	ND	D
nasal medicine	Beclomethasone (ratioAllerg® nasal spray)	15% (v/v)	D	D	ND	D
	Budesonide (Budes nasal spray)	15% (v/v)	D	D	ND	D
	Dexamethasone (Solupen® sine nasal spray)	15% (v/v)	D	D	ND	D
	Flunisolide (Syntaris® nasal spray)	15% (v/v)	D	D	ND	D
	Fluticasone (Otri-Allergie nasal spray)	15% (v/v)	D	D	ND	D
	Mometasone (MometaHEXAL® nasal spray)	15% (v/v)	D	D	ND	D
	Triamcinolone acetonide (NASACORT® nasal spray)	15% (v/v)	D	D	ND	D
	Xylometazolinhydrochlorid (Olynth® 0,1 % nasal spray)	15% (v/v)	D	D	ND	D
	Luffa opperculata D4 (Luffa nasal drops)	15% (v/v)	D	D	ND	D
	Phenylephrine (Vibrocil® nasal spray)	15% (v/v)	D	D	ND	D
	Oxymetazoline (Nasivin® nasal spray)	15% (v/v)	D	D	ND	D
	Sodium chloride (Redcare nasal spray)	15% (v/v)	D	D	ND	D
throat lozenges	Benzocaine (Dolo-Dobendan® lozenges)	1 mg/ml	D	D	ND	D
antimicro-bials	Azithromycin	1.11 mg/dl	D	D	ND	D
	Ceftriaxone	84 mg/dl	D	D	ND	D
	Levofloxacin	3.6 mg/dl	D	D	ND	D
	Meropenem	33.9 mg/dl	D	D	ND	D
	Mupirocin	0.15 mg/dl	D	D	ND	D
	Tobramycin	3.3 mg/dl	D	D	ND	D

Table 18: Interference of human blood with the res4plex direct RT-PCR test. Five replicates of artificial nasopharyngeal matrix with indicated concentrations of human blood were tested.

Target	SARS-CoV-2	Influenza A	Influenza B	RSV A	RSV B
Human Blood 2% (v/v)	5/5 detected	0/5 detected	5/5 detected	5/5 detected	5/5 detected
Human Blood 1% (v/v)	5/5 detected	5/5 detected	5/5 detected	5/5 detected	5/5 detected
Human Blood 0.5% (v/v)	5/5 detected	5/5 detected	5/5 detected	5/5 detected	5/5 detected
Human Blood 0.2% (v/v)	5/5 detected	5/5 detected	5/5 detected	5/5 detected	5/5 detected

19.4 Inclusivity

For inclusivity analysis, an in-silico analysis was performed. For this purpose, all available SARS-CoV-2, influenza A, influenza B, and RSV A/B sequences in the GISAID and the NCBI GenBank database were aligned to the assay primers and probes.

For influenza A inclusivity analysis, a total of 196,017 gene sequences from GISAID and 96,480 from NCBI GenBank were analysed consisting of H1N1 (including pdm09), H1, H3N2, H3, H5N1, H5, H5 other than H5N1, H7N9, H7 other than H7N9, H7, H9N2, H9 other than H9N2, H9, H2 and unclassified sublineages of influenza A. To analyse the inclusivity of a new influenza A primer in the res4plex *direct* RT-PCR V02 a total of 1013 influenza A H3N2 and 1514 influenza A H1N1 sequences submitted to the GISAID database from 01.11.2023 to 31.10.2024 were downloaded and aligned with the new primer.

For influenza B inclusivity analysis, a total of 34,011 gene sequences from GISAID (consisting of 19,792 Victoria strains, 10,854 Yamagata strains, and 3,365 unclassified sublineages) and 10,357 gene sequences from NCBI GenBank (consisting of 1,833 Victoria strains, 2,239 Yamagata strains, and 6,285 unclassified sublineages) were analysed.

For RSV A inclusivity analysis, a total of 3,477 gene sequences from GISAID and 2,545 from NCBI GenBank were analysed. For RSV B, a total of 3,069 from GISAID and 1541 from NCBI GenBank were analysed.

Since the res4plex *direct* RT-PCR test utilizes the US-CDC-N1 and Charite-E primer and probe set, "Common Primer Check for High Quality Genomes 2023-07-18" analysis of GISAID is referred for the inclusivity data. 280,945 gene sequences were analysed.

The frequency of sequences with an exact match was more than 90% for all assay primers and probes. Moreover, the frequency of identical primer 3' ends (first 5 nucleotides of 3' end position) was more than 97% for all assay primers and probes.

19.5 Competitive Interference

To analyse competitive interference, one test analyte was applied at the highest possible concentration (10^6 cp/ml), whereas the other test analytes were applied in a low concentration (3x LoD). If these produced negative results for analytes at 3x LoD, lower concentrations of each spiking analyte (10^5 cp/ml) were tested. All possible combinations of analytes were tested in triplicates.

In most cases, a high concentration of spiking analyte up to 10^6 cp/ml did not affect the detection of other analytes present at a low concentration (3x LoD). However, there were some exceptions. In the presence of 10^6 cp/ml SARS-CoV-2, 1 out of 3 influenza A samples and 2 out of 3 RSV B samples at 3x LoD were not detectable. Similarly, in the presence of 10^6 cp/ml influenza A, 1 out of 3 RSV A samples at 3x LoD could not be detected (see Table 19).

In conclusion, these data showed that there is potential for competitive interference of influenza A, or RSV B at low concentration (~3x LoD) when SARS-CoV-2 concentration is ≥10^6 RNA cp/mL. In addition, there is potential for competitive interference of RSV A at low concentration (~3x LoD) when influenza A concentration is ≥10^6 RNA cp/mL. As a result of these findings, it is recommended that the detection of SARS-CoV-2 should not exclude the possibility of a co-infection with influenza A, or RSV B. Similarly, the detection of influenza A should not exclude the possibility of a co-infection with RSV A.

Table 19: Competitive interference. Positivity lower than 100% is highlighted in red.

Sample	Analyte	Positive Cells	Total	% Positivity
SARS-CoV-2 at 1000 cp/µL with 3x LoD Influenza A	Influenza A	2	3	66.7
SARS-CoV-2 at 100 cp/µL with 3x LoD Influenza A	Influenza A	3	3	100.0
SARS-CoV-2 at 1000 cp/µL with 3x LoD Influenza B	Influenza B	3	3	100.0
SARS-CoV-2at 1000 cp/µL with 3x LoD RSVA	RSV A	3	3	100.0
SARS-CoV-2 at 1000 cp/µL with 3x LoD RSV B	RSV B	1	3	33.3
SARS-CoV-2 at 100 cp/µL with 3x LoD RSV B	RSV B	3	3	100.0
Influenza A at 1000 cp/µL with 3x LoD SARS-CoV-2	SARS-CoV-2	3	3	100.0
Influenza A at 1000 cp/µL with 3x LoD Influenza B	Influenza B	3	3	100.0
Influenza A at 1000 cp/µL with 3x LoD RSVA	RSV A	2	3	66.7
Influenza A at 100 cp/µL with 3x LoD RSVA	RSV A	3	3	100.0
Influenza A at 1000 cp/µL with 3x LoD RSVB	RSV B	3	3	100.0
Influenza B at 1000 cp/µL with 3x LoD SARS-CoV-2	SARS-CoV-2	3	3	100.0
Influenza B at 1000 cp/µL with 3x LoD Influenza A	Influenza A	3	3	100.0
Influenza B at 1000 cp/µL with 3x LoD RSVA	RSV A	3	3	100.0
Influenza B at 1000 cp/µL with 3x LoD RSVB	RSV B	3	3	100.0
RSVA at 1000 cp/µL with 3x LoD SARS-CoV-2	SARS-CoV-2	3	3	100.0
RSVA at 1000 cp/µL with 3x LoD Influenza A	Influenza A	3	3	100.0
RSVA at 1000 cp/µL with 3x LoD Influenza B	Influenza B	3	3	100.0
RSVB at 1000 cp/µL with 3x LoD SARS-CoV-2	SARS-CoV-2	3	3	100.0
RSVB at 1000 cp/µL with 3x LoD Influenza A	Influenza A	3	3	100.0
RSVB at 1000 cp/µL with 3x LoD Influenza B	Influenza B	3	3	100.0

19.6 Precision

The analytical performance parameter precision is derived from repeatability and reproducibility. For precision estimation, negative and positive samples at 3x LoD were analysed. The agreement between different variation parameters (lot, instruments, operator) was evaluated (see Table 20).

Table 20: Precision variation parameters

Experiment	Day	Lot	Instrument	Operator	3x LoD (# of replicates)	negative samples (# of replicates)
1	Day 1	Lot #1	Instrument 1 (CFX Opus)	Operator #1	12	1
2	Day 1	Lot #1	Instrument 2 (CFX Opus)	Operator #2	12	1
3	Day 2	Lot #2	Instrument 3 (LC480 II)	Operator #1	20	1

The coefficient of variation (CV) was calculated using the following formula:

$$CV = \frac{standard\ deviation}{mean} * 100\%$$

Depending on the analyte the intra-assay CV values ranged between 0.57% and 1.15%, and the inter-assay CV values ranged between 1.37% and 3.87% (see Table 21).

Table 21: Intra- and Inter-assay CV values. The intra-assay CV was calculated from the Ct values of experiment 3 and the inter-assay CV was calculated from Ct values of all three experiments.

Analyte (3x LoD)	Intra-assay CV (%)	Inter-assay CV (%)
SARS-CoV-2	0.96	1.25
Influenza A H1N1	0.57	3.87
Influenza A H3N2	0.93	3.61
Influenza B	1.04	2.96
RSV A	1.14	2.10
RSV B	1.15	2.80

19.7 Carryover/Cross-contamination

A run with 12 alternating columns of high positive and negative samples in one plate was performed. Positive samples contained SARS-CoV-2 RNA at a concentration of 1.15 x 10^5 TCID50/ml. There were no false negative and no false positive test results.

19.8 Whole System Failure Rate

Out of 106 tested samples that have been spiked with SARS-CoV-2 RNA at 3x LoD, 105 gave a positive result and 1 gave a negative result. Thus, the detection rate was ≥ 99%.

19.9 References

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