altona DIAGNOSTICS

Instructions for use

FlexStar®
SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5

02/2022 EN

Respiratory

FlexStar®

FlexStar®

SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5

For use with

LightCycler® 480 Instrument II (Roche)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Dx System (Bio-Rad)

CFX96™ Deep Well Dx System (Bio-Rad)

CE

IVD

REF FS0021515

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02 2022

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MAN-FS0021510-EN-S01

Content

1. About these instructions for use

Throughout this manual, the terms CAUTION and NOTE have the following meanings:

CAUTION

Highlights operating instructions or procedures which, if not followed correctly, may result in personal injury or impact product performance. Contact altona Diagnostics technical support for assistance.

NOTE

Information is given to the user that is useful but not essential to the task at hand.

Read the instructions for use carefully before using the product.

2. Intended use

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus specific RNA in human respiratory swab specimens. The SARS-CoV-2 detection is based on the parallel detection of the E gene of lineage B-beta coronavirus (including SARS-CoV-2) and the S gene of SARS-CoV-2.

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is intended to be used as an aid for diagnosis of SARS-CoV-2 and influenza virus infection.

The results generated with the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 have to be interpreted in conjunction with other clinical and laboratory findings.

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is intended for use by professional users trained in molecular biological techniques and *in vitro* diagnostic procedures.

3. Product content

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 contains the following components:

Table 1: Kit components

Lid color	Component	Number of tubes	Nominal volume [µl/tube]
Blue	Detection Mix[1)]	8	240
Red	PC[2)]	2	250
White	NTC[3)]	2	250

[1)] Contains biological material of animal origin

[2)] Positive Control [lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and influenza virus specific RNA]

[3)] No Template Control (negative control)

CAUTION

Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, product performance could be compromised.

4. Storage

- The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is shipped on dry ice. The product components should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics technical support for assistance (see chapter 15. Technical assistance).
- All components should be stored at -25 °C to -15 °C upon arrival.
- Repeated thawing and freezing of the Detection Mix component should be avoided, as this might affect the performance of the product.
- Repeated thawing and freezing of the Positive Control (PC) and No Template Control (NTC) (more than 4 times) should be avoided, as this might affect the performance of the product.
- Storage at room temperature (max. +30 °C) should not exceed a period of 2 hours.
- Protect the Detection Mix component from light.

CAUTION

Improper storage conditions could compromise product performance.

CAUTION

Do not exceed thaw-freeze-sequence and handling durations specified in these instructions for use, as this could compromise product performance.

CAUTION

Do not use products beyond the expiration date. The use of expired products could compromise product performance.

5. Background information

SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive sense, single stranded RNA virus belonging to the family *Coronaviridae*, genus betacoronavirus, subgenus lineage B.

SARS-CoV-2 emerged in the Wuhan region of China in December 2019 and has spread worldwide within 2 months. The virus was initially termed as 2019-nCoV (novel Coronavirus) and renamed as SARS-CoV-2 by the “International Committee on Taxonomy of Viruses”, on 11.02.2020. At the same time the WHO named the disease, caused by SARS-CoV-2, COVID-19. Considering the rapid escalation and propagation of COVID-19 worldwide, the WHO characterized the outbreak as a pandemic on 12.03.2020.

SARS-CoV-2 is highly contagious and transmitted via aerosols and droplets and causes acute respiratory infections with flu-like symptoms. Mainly, but not exclusively, in elderly people and persons with pre-existing illness, infection with SARS-CoV-2 can lead to severe and life-threatening disease. Cases of asymptomatic infection, mild illness, severe illness, and deaths have been reported [1,2].

Influenza virus

Influenza, commonly referred to as the flu, is an infectious disease caused by RNA viruses of the family *Orthomyxoviridae* (influenza viruses) [3,4]. Influenza viruses are characterized by the continuous change of their major surface antigens hemagglutinin (H) and neuraminidase (N) (antigenic drift) [5]. They infect birds and mammals via aerosols [6]. Human influenza A and influenza B viruses cause severe infections predominantly of the respiratory tract with fever and coughs as the most frequent symptoms. In more serious cases, influenza causes pneumonia, which can be fatal particularly for children and elderly people [7].

NOTE

Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.

6. Product description

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is an *in vitro* diagnostic test. Used in combination with the FlexStar® (RT-)PCR Amplification Mix 1.5 it allows the qualitative detection and differentiation of lineage B-beta coronaviruses (lineage B-βCoV, E gene), SARS-CoV-2 (S gene) and influenza (A+B) virus specific RNA in human respiratory swab specimens.

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is based on real-time RT-PCR technology, utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and influenza (A+B) specific target sequences and fluorescently labeled target specific probes for the detection of the amplified cDNA.

In addition to the lineage B-βCoV, SARS-CoV-2 and influenza virus RNA specific amplification and detection systems the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 includes oligonucleotides for the amplification and detection of the internal control (IC, AltoStar® Internal Control 1.5). The IC is automatically added at the beginning of the nucleic acid purification procedure on the AltoStar® Automation System AM16 (in the following summarized as AltoStar® AM16). For details refer to the instructions for use of the AltoStar® Internal Control 1.5.

Probes specific for lineage B-βCoV (E gene) RNA are labeled with the fluorophore ROX™, probes specific for SARS-CoV-2 (S gene) RNA are labeled with the fluorophore Cy5 and probes specific for influenza (A+B) virus RNA are labeled with the fluorophore FAM™, respectively. The probe specific for the IC is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of lineage B-βCoV (E gene), SARS-CoV-2 (S gene), influenza (A+B) viruses and the IC in the corresponding detection channels of the real-time PCR instrument.

6.1 Components

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 contains enough reagents for 384 reactions. The product consists of the following components:

- Detection Mix[1)]
- PC[2)]
- NTC[3)]

[1)] Contains biological material of animal origin

[2)] Positive Control [lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and influenza virus specific RNA]

[3)] No Template Control (negative control)

Except for the DNA polymerase and the reverse transcriptase, which are included in the FlexStar® (RT-)PCR Amplification Mix 1.5, the Detection Mix component contains all reagents (PCR buffer, magnesium salt, primers and probes) to allow detection and differentiation of lineage B-βCoV (E gene) and SARS-CoV-2 (S gene) and detection of influenza (A+B) virus specific RNA, as well as of IC specific RNA.

The PC contains lineage B-βCoV (E gene) and SARS-CoV-2 (S gene) as well as influenza virus specific RNA. It is used to verify the functionality of the lineage B-βCoV, SARS-CoV-2 and influenza virus specific amplification and detection systems.

The NTC contains neither lineage B-βCoV, SARS-CoV-2, nor influenza (A+B) virus specific RNA but does contain the IC template. The NTC is used as negative control for the lineage B-βCoV (E gene), the SARS-CoV-2 (S gene) and the influenza (A+B) virus specific real-time PCR and indicates possible contamination of the Detection Mix component.

6.2 Real-time PCR instruments

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 was developed and validated to be used with the following real-time PCR instruments:

- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Dx System (Bio-Rad)
- CFX96™ Deep Well Dx System (Bio-Rad)

NOTE

Ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

6.3 Sample types

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 has been validated for use with the following sample type:

- Human respiratory swab specimen

CAUTION

Do not use other sample types! The use of other sample types could compromise product performance.

7. Material required but not provided

The following additional instruments and consumables are required for use of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 but not provided with this product:

- Appropriate real-time PCR instrument (see chapter 6.2 Real-time PCR instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 9.2 Sample preparation)
- Vortex mixer
- Centrifuge (e.g. desktop centrifuge) for centrifugation of kit reagents
- Centrifuge for centrifugation of PCR plates
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

Reagents required but not included in the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5:

- FlexStar® (RT-)PCR Amplification Mix 1.5 (Order No. FS0011515)
- AltoStar® Internal Control 1.5 (Order No. IC15-16/IC15-46)

8. Warnings, precautions and limitations

- Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, product performance could be compromised.
- Improper storage conditions could compromise product performance.
- Do not exceed thaw-freeze-sequence and handling durations specified in these instructions for use, as this could compromise product performance.
- Do not use products beyond the expiration date. The use of expired products could compromise product performance.
- Do not use other sample types! The use of other sample types could compromise product performance.
- Improper handling of product components and samples may cause contamination and could compromise product performance:
    - Do not interchange vial or bottle caps.
    - Store positive and/or potentially positive material separated from the kit components.
    - Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
    - Always dispose gloves after handling positive and/or potentially positive material.
    - Do not open the PCR plates and/or tubes post amplification.
- Do not mix components from different FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 lots, as this could compromise product performance.
- Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.
- The presence of PCR inhibitors could cause false negative or invalid results.
- Disposal of hazardous and biological waste shall comply with local and national regulations to avoid environmental contamination.
- Storage of eluates under wrong conditions may lead to degradation of the lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and/or influenza virus target sequences and could compromise product performance.

- A lack of centrifugation of the product components after thawing may cause contamination with reagent residues in the lids and could compromise product performance.
- Do not exceed the PCR mix storage time, as this could compromise product performance.
- As with any diagnostic test, results shall be interpreted in consideration of all clinical and laboratory findings.
- In case the sample contains other pathogens than lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and/or influenza virus, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.
- Potential mutations within the target regions of the lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and/or influenza virus genome covered by primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.

9. Procedure

CAUTION

Improper handling of product components and samples may cause contamination and could compromise product performance:

- Do not interchange vial or bottle caps.
- Store positive and/or potentially positive material separated from the kit components.
- Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
- Always dispose gloves after handling positive and/or potentially positive material.
- Do not open the PCR plates and/or tubes post amplification.

CAUTION

Do not mix components from different FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 lots, as this could compromise product performance.

9.1 Sample collection, handling and storage

Commercially available dacron fiber- or polyester-tipped swabs with plastic shafts have to be used for sample collection. Dry swabs must be resuspended in universal transport medium (e.g. UTM® from Copan). Calcium alginate swabs, swabs with wooden shafts and/or cotton tips as well as swabs collected in agar gel must not be used. Transport should occur following the local and national instructions for the transport of biological material.

Before use respiratory swabs resuspended in UTM® should not be stored for more than 48 hours at room temperature (+20 °C to + 25 °C), 5 days at +2 °C to +8 °C or 2 months at -25 °C to -15 °C.

CAUTION

Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

NOTE

Frozen storage of samples does not compromise kit performance. When working with frozen samples, make sure samples are completely thawed and properly mixed before use.

NOTE

Do not use calcium alginate swabs, since this might lead to incorrect or invalid results due to PCR inhibition.

NOTE

Do not use swabs with wooden shafts and/or cotton tips or swabs with agar gel as transport medium, since remains of wood, cotton and/or agar may interfere with the sample transfer on the AltoStar® AM16 and the samples will not be processed.

9.2 Sample preparation

Extracted RNA is the starting material for the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5. The quality of the extracted RNA has a profound impact on the performance of the product.

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 was validated with human respiratory swab specimens using the AltoStar® AM16 in combination with the AltoStar® Purification Kit 1.5.

After completion of nucleic acid extraction using the AltoStar® AM16, the eluates in the unsealed eluate plate are stable at room temperature (max. +30 °C) for a total of 4 hours.

The eluates in a sealed eluate plate can be stored at +2 °C to +8 °C for up to 24 hours before the start of a reaction setup. For detailed information regarding the sealing of eluate plates, refer to the instructions for use of the AltoStar® Purification Kit 1.5.

Alternative nucleic acid extraction systems and kits might also be appropriate. However, the suitability of the nucleic acid extraction procedure for use with the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 has to be validated by the user.

CAUTION

Do not use other sample types! The use of other sample types could compromise product performance.

CAUTION

Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

CAUTION

The presence of PCR inhibitors could cause false negative or invalid results.

CAUTION

Disposal of hazardous and biological waste shall comply with local and national regulations to avoid environmental contamination.

CAUTION

Storage of eluates under wrong conditions may lead to degradation of the lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and/or influenza virus target sequences and could compromise product performance.

For additional information and technical support regarding pre-treatment and sample preparation, contact altona Diagnostics technical support (see chapter 15. Technical assistance).

9.3 Master mix setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is configured for use with the FlexStar® (RT-)PCR Amplification Mix 1.5 and the AltoStar® Internal Control 1.5, which allows to control the sample preparation procedure (nucleic acid extraction) and the subsequent RT-PCR.

- ► The IC is automatically added at the beginning of the nucleic acid purification procedure on the AltoStar® AM16.
- ► When using other nucleic acid extraction methods, the IC has to be added during the lysis step either manually or automatically by the respective instrument.
- ► No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample. The IC should always be added to the sample/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 50 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 30 µl of IC per sample must be added into the sample/lysis buffer mixture.
- ► Set up the master mix according to the following pipetting scheme:

Table 2: Pipetting scheme (master mix setup)

Number of reactions (rxns)	1	12
Detection Mix	5 µl	60 µl
Amplification Mix	15 µl	180 µl
Volume master mix	**20 µl**	**240 µl**

CAUTION

A lack of centrifugation of the product components after thawing may cause contamination with reagent residues in the lids and could compromise product performance.

9.4 Reaction setup

- ► Pipette 20 µl of the master mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (PC or NTC).

Table 3: Pipetting scheme (reaction setup)

Reaction setup	
Master mix	20 µl
Sample or control	10 µl
Total volume	**30 µl**

- ► Make sure that at least 1 PC and 1 NTC is used per run.
- ► Thoroughly mix the samples and controls with the master mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor and the reaction tubes in an appropriate centrifuge for 30 seconds at approximately 1,000 x g (~ 3,000 rpm).
- ► The NTC does already contain the IC template in the correct concentration.

After completion of the PCR mix setup the RT-PCR mix in a sealed PCR plate is stable at room temperature (max. +30 °C) for max. 30 minutes.

CAUTION

Do not exceed the PCR mix storage time, as this could compromise product performance.

10. Programming the real-time PCR instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 on specific real-time PCR instruments, contact altona Diagnostics technical support (see chapter 15. Technical assistance).

10.1 Settings

► Define the following settings:

Table 4: Run settings

Settings	
Reaction volume	30 µl
Ramp rate	Default
Passive reference	None

10.2 Fluorescence detectors (dyes)

► Define the fluorescence detectors (dyes):

Table 5: Fluorescence detectors

Target	Detector name	Reporter	Quencher
Lineage B-βCoV specific RNA	E gene	ROX™	(None)
SARS-CoV-2 specific RNA	S gene	Cy5	(None)
Influenza (A+B) virus specific RNA	Flu	FAM™	(None)
Internal Control	IC	JOE™	(None)

10.3 Temperature profile and dye acquisition

► Define the temperature profile and dye acquisition:

Table 6: Temperature profile and dye acquisition

	Stage	Cycle repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse transcription	Hold	1	-	52	05:00
Denaturation	Hold	1	-	95	00:05
Amplification	Cycling	45	-	95	00:05
			Yes	58	00:25

11. Data analysis

For basic information regarding data analysis on specific real-time PCR instruments, refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 on different real-time PCR instruments, contact altona Diagnostics technical support (see chapter 15. Technical assistance).

11.1 Validity of diagnostic test runs

11.1.1 Valid diagnostic test run

A diagnostic test run is **valid**, if the following control conditions are met:

Table 7: Control conditions for a valid test run

Control ID	Detection channel			
	ROX™	Cy5	FAM™	JOE™
Positive Control [lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and influenza virus]	+	+	+	Not applicable
Negative control	-	-	-	+

11.1.2 Invalid diagnostic test run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

11.2 Interpretation of results

CAUTION

As with any diagnostic test, results shall be interpreted in consideration of all clinical and laboratory findings.

11.2.1 Qualitative analysis

Table 8: Result interpretation

Detection channel				Result interpretation
ROX™ (E gene)	Cy5 (S gene)	FAM™ [Influ-enza (A+B)]	JOE™ (IC)	
+	+	-	+/-*	Lineage B-βCoV and SARS-CoV-2 specific RNA detected.
+	-	-	+/-*	Only lineage B-βCoV specific RNA detected.**
-	+	-	+/-*	Only SARS-CoV-2 specific RNA detected.**
-	-	+	+/-*	Only influenza (A and/or B) virus RNA detected.
+	-	+	+/-*	Lineage B-βCoV and influenza (A and/or B) virus specific RNA detected.
-	+	+	+/-*	SARS-CoV-2 and influenza (A and/or B) virus specific RNA detected.
+	+	+	+/-*	Lineage B-βCoV, SARS-CoV-2 and influenza (A and/or B) virus specific RNA detected.
-	-	-	+	Neither lineage B-βCoV nor SARS-CoV-2, nor influenza (A and/or B) virus specific RNA detected. The sample does not contain detectable amounts of lineage B-βCoV, SARS-CoV-2 or influenza virus specific RNA.
-	-	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the IC in the JOE™ detection channel is not required for positive results in the ROX™ and/or the Cy5 and/or the FAM™ detection channel. A high lineage B-βCoV (target E gene) and/or SARS-CoV-2 (target S gene) and/or influenza (A+B) RNA load in the sample can lead to reduced or absent IC signals.

** Detection in only one of the two respective detection channels for lineage B-βCoV (E gene) and SARS-CoV-2 (S gene) might be due to low viral RNA concentration close to the limit of detection or due to mutation of one of the two target sequences.

12. Performance evaluation

The performance of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 was evaluated using the 1st WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146), influenza A virus material (influenza A H3N2 strain Wisconsin/67/05) and influenza B virus material (strain Florida/04/06) commercially available.

12.1 Respiratory swabs

12.1.1 Analytical sensitivity

For the determination of the limit of detection (LoD) a dilution series of the 1st WHO International Standard for SARS-CoV-2 RNA, influenza A virus material (influenza A H3N2 strain Wisconsin/67/05) and influenza B virus material (strain Florida/04/06) commercially available diluted in universal transport medium (UTM®, Copan) containing simulated nasal matrix [5 % w/v Mucin, 5 % v/v blood, 0.8 % v/v NaCl (95 % saline) and 0.00002 % w/v human genomic DNA (510k Submission for BD MAX™ MRSA XT assay; accession number: K133605)] was generated.

A dilution series of the 1st WHO International Standard for SARS-CoV-2 RNA ranging from 1.00E+04 IU/ml to 5.00E-01 IU/ml was tested. For influenza A virus and influenza B virus, a dilution series ranging from 2.00E+03 copies/ml to 1.00E+01 copies/ml was tested.

Each dilution was tested in 8 replicates in 3 different runs (total n = 24 per dilution) using combinations of:

- 3 FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 lots
- 3 FlexStar® (RT-)PCR Amplification Mix 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

For each virus the data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 9: PCR results used for the calculation of the analytical sensitivity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for SARS-CoV-2 (S gene)

Concentration [IU/ml]	N [total]	N [positive]	Hit rate [%]
1.00E+04	24	24	100
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	24	100
1.00E+02	24	24	100
3.16E+01	24	10	42
1.00E+01	24	4	17
3.16E+00	24	5	21
1.00E+00	24	1	4
5.00E-01	24	1	4

The LoD of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for the detection of SARS-CoV-2 (S gene) in UTM® is 201 IU/ml (95 % confidence interval: 107–501 IU/ml).

Table 10: PCR results used for the calculation of the analytical sensitivity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for SARS-CoV-2 (E gene)

Concentration [IU/ml]	N [total]	N [positive]	Hit rate [%]
1.00E+04	24	24	100
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	24	100
1.00E+02	24	23	96
3.16E+01	24	10	42
1.00E+01	24	4	17
3.16E+00	24	2	8
1.00E+00	24	1	4
5.00E-01	24	1	4

The LoD of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for the detection of SARS-CoV-2 (E gene) in UTM® is 226 IU/ml (95 % confidence interval: 124–545 IU/ml).

Table 11: PCR results used for the calculation of the analytical sensitivity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for influenza A virus

Concentration [copies/ml]	N [total]	N [positive]	Hit rate [%]
2.00E+03	24	24	100
1.00E+03	24	24	100
7.50E+02	24	24	100
5.00E+02	24	23	96
2.50E+02	24	22	92
1.00E+02	24	17	71
5.00E+01	24	9	38
2.50E+01	24	6	25
1.00E+01	24	1	4

The LoD of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for the detection of influenza A virus in UTM® is 341 copies/ml (95 % confidence interval: 230–611 copies/ml).

Table 12: PCR results used for the calculation of the analytical sensitivity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for influenza B virus

Concentration [copies/ml]	N [total]	N [positive]	Hit rate [%]
2.00E+03	24	24	100
1.00E+03	24	24	100
7.50E+02	24	24	100
5.00E+02	24	24	100
2.50E+02	24	19	79
1.00E+02	24	3	13
5.00E+01	24	6	25
2.50E+01	24	5	21
1.00E+01	24	1	4

The LoD of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for the detection of influenza B virus in UTM® is 432 copies/ml (95 % confidence interval: 286–780 copies/ml).

12.1.2 Analytical specificity

The analytical specificity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant SARS-CoV-2 and influenza virus genotypes will be detected.

For the verification of the analytical specificity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 the following experiments were performed (see chapters 12.1.2.1 Negative samples to 12.1.2.3 Cross-reactivity).

12.1.2.1 Negative samples

30 SARS-CoV-2, influenza A virus and influenza B virus negative respiratory swab samples from individual donors were tested with the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5. All (30 out of 30) samples were tested negative for SARS-CoV-2, influenza A virus and influenza B virus specific RNA and positive for the IC. The analytical specificity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 for respiratory swab samples is ≥ 95 %.

12.1.2.2 Interfering substances

To evaluate the influence of potentially interfering endogenous and exogenous substances on the performance of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5, selected substances were spiked in UTM® containing SARS-CoV-2, influenza A virus and influenza B virus at a final concentration of the 3 x LoD (6.03E+02 IU/ml, 1.02E+03 copies/ml and 1.29E+03 copies/ml, respectively) and in UTM® not containing SARS-CoV-2, influenza A virus and influenza B virus.

Results obtained for samples containing potentially interfering substances were compared to results generated for UTM® containing no spiked interferent. Each sample was processed in 3 replicates.

No interference was observed for samples containing elevated levels of:

- Endogenous substances
  - Human genomic DNA
  - Human whole blood
  - Mucin
- Exogenous substances
  - Antiallergic nasal spray (containing beclomethasone dipropionate)
  - Decongestant nasal spray (containing xylometazolinhydrochloride and dexpanthenol)
  - Mupirocin
  - Zanamivir

CAUTION

The presence of PCR inhibitors could cause false negative or invalid results.

12.1.2.3 Cross-reactivity

The analytical specificity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 with respect to cross-reactivity with other pathogens than SARS-CoV-2, influenza A virus and influenza B virus was evaluated by testing:

- Pathogens related to SARS-CoV-2 and influenza viruses
- Pathogens causing similar symptoms as an infection with SARS-CoV-2 or influenza viruses

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 did not cross-react with any of the following pathogens:

- Adenovirus
- *Bordetella pertussis*
- *Bordetella parapertussis*
- *Chlamydia pneumoniae*
- Enterovirus
- *Haemophilus influenzae*
- Human coronavirus 229E
- Human coronavirus NL63
- Human coronavirus OC43
- Human Metapneumovirus (hMPV)
- *Legionella pneumophila*
- MERS-coronavirus
- *Moraxella catarrhalis*
- *Mycoplasma pneumoniae*
- Parainfluenza virus 1–4
- *Pneumocystis jirovecii*
- Respiratory syncytial virus A
- Respiratory syncytial virus B
- Rhinovirus
- *Streptococcus pneumoniae*

CAUTION

In case the sample contains other pathogens than lineage B-βCoV (E gene), SARS-CoV-2 (S gene) and/or influenza virus, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.

12.1.3 Inclusivity

Specificity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 with respect to the detection of different SARS-CoV-2 variants and different strains of influenza virus is foremost ensured by the selection of the primers and probes. To verify that the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 allows the detection of different SARS-CoV-2 variants and different strains of influenza virus, the following variants/strains were tested (refer to tables 13 and 15).

Table 13: Tested SARS-CoV-2 lineages

Variant (lineage)	ROX™ channel (E gene)	Cy5 channel (S gene)	VIC™ channel (IC)
BetaCoV/Munich/ChVir984/2020 (wild type)	+	+	+
2019-nCoV/Italy-INMI1 (wild type)	+	+	+
Alpha (B.1.1.7)	+	+	+
Beta (B.1.351)	+	+	+
Delta (B.1.617.2)	+	+	+
Gamma (P.1)	+	+	+

Table 14: Inclusivity [*in silico* analysis for 2,993,884 whole genome sequences of SARS-CoV-2 published via GISAID e.V. (www.gisaid.org) as of October 10, 2021 and 518,615 whole genome sequences published via National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) as of October 10, 2021 for the E gene and the S gene target: FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5]

3,512,499 whole genome sequences		Sequences showing 100 % homology	Sequences showing mismatches (number of mismatches)
E gene	Forward primer	3,505,942	6,549 (1) 8 (2)
	Reverse primer	3,509,461	3,032 (1) 5 (2) 1 (3)**
	Probe	3,510,308	2,183 (1) 4 (2)
S gene	Forward primer	3,491,594	20,728 (1) 160 (2)
	Reverse primer	3,490,361	22,061 (1) 77 (2)
	Probe	3,498,798	13,653 (1) 44 (2) 3 (3) 1 (4)*

* The sequence (accession ID EPI_ISL_415593, GISAID) showed 4 mismatches in the S gene probe binding site. This sequence was published on March 10, 2020 originating from Washington, USA. Since then, none of the published sequences showed that many mismatches again. The sequence was commented by the authors “Caution. Stretches of NNNs (1.74 % of overall sequence)”, indicating not ideal sequencing quality, the impact on the S gene specific oligonucleotides has therefore not been investigated.

** The sequence (accession MW584978.1) showed 3 mismatches in the E gene reverse primer binding site. This sample was collected on April 03, 2020 and published on February, 2021 originating from Cleveland, USA. Since then, none of the published sequences showed that many mismatches again.

Depending on the position, mutation events leading to ≤ 2 mismatch/es in a single oligonucleotide sequence are very unlikely to have any significant negative effect on the performance of the assay. All such sequences (≤ 2 mismatch/es) tested in wet lab experiments in the cause of the post market surveillance activities for the RealStar®, FlexStar® and AltoStar® kits for detection of SARS-CoV-2 so far confirmed that the performance was not affected by such mutations. With the exception of one unique sequence none of the other analyzed sequences showed mismatches in more than one oligonucleotide and none of the mismatching sequences showed mismatches with both specific detection systems (E gene and S gene), hence reactivity of the specific oligonucleotides included in the RealStar®, FlexStar® and AltoStar® kits for detection of SARS-CoV-2 is not expected to be affected.

Table 15: Tested influenza A and influenza B virus strains

Subtype/strain	FAM™ channel (influenza A and B virus)	VIC™ channel (IC)
Influenza A virus, subtype H1N1 (New Caledonia/20/98)	+	+
Influenza A virus, subtype H1N1pdm09 (A/NY/02/2009)	+	+
Influenza A virus, subtype A/H3N2 drift variant (A/Sachsen/2/2015)	+	+
Influenza A virus, subtype H5N1 (A/Anhui/1/05)	+	+
Influenza B virus (B/Colorado/6/2017, B-Victoria lineage)	+	+
Influenza B virus (B/Phuket/3073/2013, B/Yamagata 16/88 lineage)	+	+

12.1.4 Precision

Precision of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 was evaluated using a panel consisting of:

- 1 SARS-CoV-2 high positive [50 x LoD (1.00E+04 IU/ml)] sample in UTM® containing simulated nasal matrix
- 1 influenza A virus high positive [50 x LoD (1.70E+04 copies/ml)] sample in UTM® containing simulated nasal matrix
- 1 influenza B virus high positive [50 x LoD (2.16E+04 copies/ml)] sample in UTM® containing simulated nasal matrix
- 1 SARS-CoV-2 low positive [3 x LoD (6.03E+02 IU/ml)] sample in UTM® containing simulated nasal matrix
- 1 influenza A virus low positive [3 x LoD (1.02E+03 copies/ml)] sample in UTM® containing simulated nasal matrix
- 1 influenza B virus low positive [3 x LoD (1.29E+03 copies/ml)] sample in UTM® containing simulated nasal matrix
- 1 SARS-CoV-2, influenza A virus and influenza B virus negative sample (UTM® containing simulated nasal matrix)

Each panel member was tested in at least 6 replicates per run.

5 runs were performed on 5 different days using combinations of:

- 3 FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 lots
- 3 FlexStar® (RT-)PCR Amplification Mix 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Repeatability (intra-run variability), inter-lot variability and reproducibility (total variability) were determined based on:

- Threshold cycle (C_q*) values for the SARS-CoV-2, influenza A virus and influenza B virus high positive samples (see tables 16 and 17)
- Threshold cycle (C_q*) values for the IC in the SARS-CoV-2, influenza A virus and influenza B virus negative samples (see table 18)

* Please note that the chosen term C_q is equivalent to the designation of C_t, which might be used by other cyclers than the CFX96™ Deep Well Dx System (Bio-Rad).

Table 16: Precision data (CV % C_q values) for SARS-CoV-2 high positive samples

	SARS-CoV-2 high positive sample (C_q in the ROX™ channel, target E gene)	SARS-CoV-2 high positive sample (C_q in the Cy5 channel, target S gene)
Intra-run variability	0.21–0.42	0.44–0.60
Inter-lot variability	0.59	0.52
Total variability	0.89	0.63

All samples tested at 3 x LoD (low positive samples) were detected positive for SARS-CoV-2 (E gene and S gene).

Table 17: Precision data (CV % C_q values) for influenza A virus and influenza B virus high positive samples

	Influenza A virus high positive sample (C_q in the FAM™ channel)	Influenza B virus high positive sample (C_q in the FAM™ channel)
Intra-run variability	0.93–1.16	0.52–1.48
Inter-lot variability	1.02	1.02
Total variability	1.48	1.69

All samples tested at 3 x LoD (low positive samples) were detected positive for influenza A virus and influenza B virus.

Table 18: Precision data (CV % C_q values) for the IC in SARS-CoV-2, influenza A virus and influenza B virus negative samples

	IC
Intra-run variability	0.23–0.35
Inter-lot variability	0.38
Total variability	0.74

12.1.5 Total failure rate

The robustness of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 was assessed by testing 30 SARS-CoV-2, influenza A virus and influenza B virus negative human respiratory swab samples from individual donors spiked with SARS-CoV-2, influenza A virus and influenza B virus to a final concentration of the 3 x LoD (6.03E+02 IU/ml of SARS-CoV-2, 1.02E+03 copies/ml of influenza A virus and 1.29E+03 copies/ml of influenza B virus). All (30 out of 30) samples were tested positive in the SARS-CoV-2 specific fluorescence detection channels (Cy5 and ROX™) and in the influenza A virus and influenza B virus specific fluorescence detection channel (FAM™).

12.1.6 Carry over

Carry over is mostly a workflow dependent risk and independent of the PCR assay used. For the AltoStar® Workflow the AltoStar® Parvovirus B19 PCR Kit 1.5 was used as exemplary model. Potential cross-contamination through carry over from high positive samples was evaluated by testing alternating parvovirus B19 high positive (1.00E+07 IU/ml) and negative samples (n = 44 each per run; 2 runs) with the AltoStar® Parvovirus B19 PCR Kit 1.5. No carry over was observed, i.e. all parvovirus B19 negative samples were tested negative.

12.1.7 Clinical performance

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 was evaluated in a comparative study with the CE-marked *ampli*Cube Respiratory Flu & SARS-CoV-2 LC (Mikrogen Diagnostik). Retrospectively, 165 individual human respiratory swab samples were tested in parallel.

The *ampli*Cube Respiratory Flu & SARS-CoV-2 LC (Mikrogen Diagnostik) was used in combination with the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) and the MagNA Pure 96 Extraction System (Roche).

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 was used in combination with the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 on the AltoStar® AM16 and the CFX96™ DW Dx.

For the qualitative analysis all samples with an invalid result for one or both assays were excluded.

Results for the remaining samples (164 for SARS-CoV-2 and 152 for influenza virus) are shown in tables 19 and 20, respectively.

Table 19: Results of the evaluation of the diagnostic sensitivity and specificity for SARS-CoV-2 in respiratory swabs

		*ampli*Cube Respiratory Flu & SARS-CoV-2 LC (Mikrogen Diagnostik)	
		POSITIVE	NEGATIVE
FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5	POSITIVE	58	1
	NEGATIVE	0	105

The diagnostic sensitivity and specificity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 compared to the *ampli*Cube Respiratory Flu & SARS-CoV-2 LC (Mikrogen Diagnostik) were 100 % (confidence interval 93.8 %–100 %) and 99.1 % (confidence interval 94.9 %–100 %), respectively.

Table 20: Results of the evaluation of the diagnostic sensitivity and specificity for influenza virus in respiratory swabs

		*ampli*Cube Respiratory Flu & SARS-CoV-2 LC (Mikrogen Diagnostik)	
		POSITIVE	NEGATIVE
FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5	POSITIVE	37	1
	NEGATIVE	0	114

The diagnostic sensitivity and specificity of the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 compared to the *ampli*Cube Respiratory Flu & SARS-CoV-2 LC (Mikrogen Diagnostik) were 100 % (confidence interval 90.1 %–100 %) and 95.3 % (confidence interval 95.3 %–100 %), respectively.

13. Disposal

Dispose of hazardous and biological waste in compliance with local and national regulations. Leftover product components and waste should not be allowed to enter sewage, water courses or the soil.

CAUTION

Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

CAUTION

Disposal of hazardous and biological waste shall comply with local and national regulations to avoid environmental contamination.

14. Quality control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is tested against predetermined specifications to ensure consistent product quality.

15. Technical assistance

For customer support, contact altona Diagnostics technical support:

e-mail: **support@altona-diagnostics.com**

phone: **+49-(0)40-5480676-0**

NOTE

Any serious incident that has occurred in relation to this product shall be reported to altona Diagnostics and the competent authority of your country.

16. Literature

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17. Trademarks and disclaimers

AltoStar®, FlexStar® (altona Diagnostics); CFX96™ (Bio-Rad); UTM® (Copan); Rotor-Gene® (QIAGEN); LightCycler® (Roche); FAM™, JOE™, ROX™, VIC™ (Thermo Fisher Scientific).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

18. Explanation of symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
GTIN	Global Trade Item Number
LOT	Batch code
CONT	Content
CAP	Cap color
REF	Catalogue number
NUM	Number
COMP	Component
	Consult instructions for use
	Contains sufficient for "n" tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
MAT	Material number

Symbol	Explanation
	Version
	Note
	Contains biological material of animal origin

19. Revision history

Table 21: Revision history

Identifier	Date of issue [month/year]	Modifications
MAN-FS0021510-EN-S01	02/2022	Initial release

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COV-FlexStar-DM-CE-EN-02_01/2021