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COVID-FLU-RSV All-in-One RT PCR

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REF **D-MBK0097** Σ 96 IVD CE

1. INTENDED USE

COVID-FLU-RSV All-in-One RT PCR is a multiplex real-time RT-PCR one-step assay for the qualitative detection of RNA from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Influenza A/B virus (Flu A/B) and Respiratory Syncytial virus A/B (RSV A/B) in naso-pharingeal and saliva samples. The test is intended for the differential diagnosis of infections by SARS-CoV-2, Influenza A/B virus and Respiratory syncytial virus in combination with other clinical and epidemiological data of patients.
Positive results are indicative for the presence of RNA from SARS-CoV-2, Flu A/B and RSV A/B. Positive results do not rule out the possibility to have co-infections caused by the presence of other viruses or bacteria.
Negative results do not exclude infections caused by SARS-CoV-2, Flu A/B, and RSV A/B and cannot be used as unique information for the clinical management of patients. Negative results must be combined with clinical observations, patient's history and epidemiological observations.
The use of the product is intended for use by well trained personnel in the techniques of real-time PCR and *in vitro* diagnostic procedures.

2. INTRODUCTION

The use of tests which allow the differential diagnosis of influenza and COVID-19 is strongly recommended at international level, especially with the arrival of the flu season [ESWI, Conclusions of ESWI's and recommendations, 2020; CDC, https://www.cdc.gov/flu/symptoms/testing.htm].
Given the similarity in terms of symptoms between seasonal flu and COVID-19, the avaiability of multiplex-type assays will be essential in order to implement effective disease control as well as clinical treatment.

3. PRINCIPLE OF THE ASSAY

COVID-FLU-RSV All-in-One RT PCR is a RT-PCR system that allows the RNA amplification and differentiation of SARS-CoV-2, human Influenza A/B viruses and the Respiratory Syncytial viruses A/B in one reaction.
Starting from the extracted RNA, a single reaction of reverse transcription and specific amplification for SARS-CoV-2, FluA/B, RSV A/B and the endogenous control **RNaseP** is carried out. The endogenous control is used to monitor the adequacy of the sample, the RNA extraction process, and the presence of PCR inhibitors. The target regions and the fluorophores used for every specific pathogen are in Table 1:

Table1: Pathogen, target region and fluorophores

Pathogen	Target region	Fluorophore
SARS-CoV-2	ORF1b/N genes	FAM
Flu A/B	M1/NS2 genes	Cal Fluor Orange 560
RSV A/B	M gene	Cal Fluor Red 610
-	RNase P gene*	Quasar 670

*Endogenous control

4. KIT CONTENT

The kit provides all the reagents required for the analysis, including the PCR negative and positive control (Table 2).

Table 2: kit content

Reagent	No vial x Volume	Cap colour
Mix 1	1 X 550 µL	Green
Mix 2	1 X 70 µL	Orange
Mix Primer/Probe	1 X 1040 µL	Blue
PCR Negative Control	1 X 100 µL	Clear
PCR Positive Control	1 X 50 µL	Red

5. REQUIRED MATERIAL NOT SUPPLIED

Disposable powder-free gloves
RNA isolation kit
Pipettes (adjustable)
Sterile pipette tips with aerosol-preventive filter
Vortex mixer
Bench microcentrifuge
Real-time PCR instrument
Consumables compatible with the real-time PCR instrument. White and clear plates, clear 8-well tubes strips, optical sealing foils and optical 8 caps tubes.
Sterile 1.5 mL tubes
Laboratory freezers

The kit is validated and can be used with the follow extraction systems and thermalcyclers:

Validated extraction methods

- QIAamp MinElute Virus spin kit (Qiagen)
- QIAamp Viral RNA Mini kit (Qiagen)
- High Pure Viral RNA/DNA Kit (Magen)
- Invisorb® Spin Virus RNA Mini Kit (Stratec)
- Total RNA Purification kit (Norgen)
- MGISP-960 platform using the Virus DNA/RNA Extraction kit (MGI)
- KingFisher™ Flex Purification System using MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Thermofisher)
- Nextractor NX48-S platform using the NX48S VIRAL NA EXTRACTION KIT (Genolution)

For use with other extraction methods and/or Real-Time PCR instruments please contact Diatheva.

Thermalcyclers

- CFX96 Biorad
- ABI7500 Applied Biosystems
- Rotor gene Q 5-plex Biorad
- Quant Studio 5 Applied Biosystems

6. ASSAY LIMITATIONS

- Samples must be collected, transported and stored using appropriate procedures and conditions. Improper sample collection, transfer, storage and processing may cause erroneus test results.
- The kit uses purified RNA as sample for the analysis. The quality of the RNA recovered from biological samples is essential for the quality of results generated with this kit.

False negative results may arise from:

- Improper sample collection
- Degradation of viral RNA during shipping/storage
- Using poor extraction method
- The presence of RT-PCR inhibitors
- Possible polimorfisms in the target regions where the sequences hybridize (primers and probes) may affect the target detection
- Failure to follow the instruction for use

False positive results may arise from:

- Cross contamination during specimen collection, handling or preparation
- RNA contamination during product handling

7. STORAGE CONDITIONS AND STABILITY OF THE REAGENTS

Upon arrival, store at -20° C. If stored at the recommended temperature all reagents are stable until the expiration date.
The performance of kit components is not affected for up to 5 freezing and thawing. If the reagents are to be used only intermittently, they should be stored in aliquots.

8. WARNING AND PRECAUTIONS

Before using the kit read the Product Information carefully and completely.
The operator should always pay attention to:

- Set up pre- and post-PCR areas. Do not share instruments or consumables (pipettes, tips, tubes, etc) between those areas;
- Store positive material separately from all other reagents and, if possible, add it to the reaction mix in a separated space;
- Do not use any reagent after the expiration date indicated on the label;
- Wear powder-free gloves during all procedures;
- Thaw all kit components and protect from light before starting the assay. After thawing, mix the components and centrifuge briefly;
- Do not substitute or mix reagents from different batches in order to maintain optimal performances;
- Include in each run at least 1 PCR Negative control and 1 PCR Positive Control;
- Minimize sample handling;
- Change gloves frequently;
- Wash the bench surfaces with 5% sodium hypochlorite;
- Use sterile disposable laboratory materials and do not re-use the tubes and tips;
- Store the reagents at the recommended temperature;

- Dispose waste in compliance with the local regulations;
- The quality of the sample preparation may influence the quality of the RT-PCR test.

9. INSTRUCTION FOR USE

9.1. SAMPLE COLLECTION

For collection methods refer to specimen collection devices manufacturer instructions.
For the collection of upper and lower respiratory samples the use of sterile collection system is recommended.
For the collection of saliva sample, the use of OMNIgen ORAL (DNAgenotec, code OME-505), Salivette® (Sarsted code 51.1534) or sterile wide mouth centrifuge tubes in polypropylene and conical bottom (eg. Falcon® 50mL) is recommended.

9.2. SAMPLE PREPARATION

The samples should be extracted according to the corresponding requirements and procedures of the viral RNA extraction kits. [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance].
The kit has been tested on RNA samples extracted using the extraction methods listed on page 2. For additional extraction methods not listed in the table please contact Diatheva. The extracted RNA samples can be stored at below -70°C, avoiding repeated freeze-thaw cycles.

9.3. SETUP REAL TIME PCR INSTRUMENT

Before preparing the reaction mix, program the real-time PCR instrument with the following thermal profile:

Table 3: Thermal profile setting

Phase	Temperature and time	Cycles
cDNA synthesis	48°C 20 min	1 X
Initial denaturation	95°C 2 min	1 X
Denaturation	95°C 10 sec	42 X**
Annealing-extension*	58°C 1 min	

*fluorescence of different fluorophores is detected during the **annealing-extension** step in the channels specified in Table 4
** using the Rotor gene Q 5-plex instrument set 40 cycles

Using the Rotor gene Q 5-plex instrument, gain optimization is required in the acquisition channels: select "Perform Optimization Before 1 st Acquisition" and place the NTC (PCR Negative Control) in the first position

Table 4: Fluorescence channel setting

Multiplex	Fluorescence reading channels			
	FAM (Green Channel)	**VIC/ Cal Fluor Orange 560 (Yellow Channel)**	**ROX/ Cal Fluor Red 610 (Orange Channel)**	**Cy5/ Quasar 670 (Red Channel)**
Target	SARS-CoV-2	Flu A/B	RSV A/B	RNaseP

- The final reaction volume is **20 µL**
- **For instruments that require it in "Run mode" or at the request "Which ramp speed do you want to use in the instrument run" select "Standard",** respective examples for QS5 and ABI 7500
- **For each target, in the respective "Quencher" box, select "None" in the requesting instruments,** i.e.: ABI 7500, QS5
- **For requesting instruments, select "None" in the function "Select dye to use as passive reference"** i.e.: ABI 7500, QS5

9.4. REACTION SETUP

All experiments must include a negative PCR control (NTC-No Template Control), containing all reaction reagents except the template, and a positive PCR control, containing all reaction reagents and the PCR Positive Control. Both controls are necessary to verify the validity of the PCR reaction as the negative control allows to determine the presence of potential contamination while the positive control allows to verify the correctness of the reaction / run set up. In the analysis phase they are also essential for setting the threshold.

- Thaw the components protect from light.
- Gently vortex Mix 1 and Mix Primer/Probe for 6 sec and centrifuge briefly. Gently vortex Mix 2 for 2 sec and centrifuge briefly.
- In one sterile tube, prepare the amplification reaction mix (Master mix) needed for each sample to be tested plus one Negative control and one Positive control, following the scheme shown in Table 5:

Table 5: Mastermix preparation

Reagent	Reagent volume for 1 reaction*
Mix 1	5 µL
Mix 2	0.625 µL
Mix Primer/Probe	9.375 µL
Total volume	15 µL

* For the analysis of more than one sample, simply multiply the volumes of Mix 1, Mix 2 and Mix Primer/Probe for the number of samples +1 (N + 1) to be tested considering the NTC and Positive Control.

- Gently vortex the tube containing the Mastermix for 6" and centrifuge briefly
- Aliquot **15 µL of Mastermix** in the PCR tubes or in the wells of the plate prepared for the experiment for each sample to test, PCR Negative Control and PCR Positive Control.
- Add **5 µL of the PCR Negative Control** in the corresponding tube/well
- In a separate area, add **5 µL of RNA samples to be analyzed and the Positive Control** in the corresponding wells in which the Mastermix has been previously aliquoted.
- Seal hermetically the PCR plate/tubes and load into the real-time PCR instrument, following the manufacturer's instructions

NOTE: It is recommended to centrifuge the tubes or the PCR plate before inserting them into the thermalcycler to eliminate air bubbles and collect all the reagents at the bottom of each well. Verify that the liquid is at the bottom of each well, otherwise centrifuge again at higher rpm for a longer time

9.5. ANALYSIS AND INTERPRETATION OF RESULTS

The analysis of the results must be performed using the PCR software of each specific instrument and referring to the manual for detailed information. Set the baseline and threshold values. Some software performs data analysis automatically, in this case it is advisable to check these settings. For manual data analysis, analyze the PCR file separately for the four fluorophores.

NOTE: for a correct result interpretation, pay attention to the amplification curves. In particular, in the amplification graph in linear phase, verify that the threshold is set to intersect the exponential phase of the PCR Positive Control fluorescence curve and above any background signal. The threshold value of different instruments may vary due to different signal intensities.

Using the instruments CFX96, ABI 7500, Rotor gene Q 5-plex, Quant Studio5 refer respectively to **appendices A, B, C, D** for the set-up of the analysis parameters.

Before interpreting the sample results it is necessary to verify the validity of the PCR analysis.
Proceed with checking the controls based on the following table:

Table 6: Reaction controls check

Control	Fluorescence Channel			
	FAM (Green Channel)	**VIC/ Cal Fluor Orange 560 (Yellow Channel)**	**ROX/ Cal Fluor red 610 (Orange Channel)**	**Cy5/ Quasar 670 (Red Channel)**
PCR Positive Control	Positive (SARS-CoV-2)	Positive (Flu A/B)	Positive (RSV A/B)	Positive (RNaseP)
PCR Negative Control (NTC)	No amplification signal	No amplification signal	No amplification signal	No amplification signal

If the run is valid, continue with the interpretation of the sample results according to the table below.
Alternatively, if the run is not valid, please repeat the test.

The following table lists an example of expected results for the kit with valid positive and negative control. The Ct cut-off values shown below were derived from product verification and validation studies. The user should determine the specific Ct cut-off values to achieve optimal performance.

Table 7: Analysis of results

Amplification				Interpretation of results
Target: SARS-CoV-2	**Target: Flu A/B**	**Target: RSV A/B**	**Target: RNaseP**	
FAM (Green Channel)	**VIC/ Cal Fluor Orange 560 (Yellow Channel)**	**ROX/ Cal Fluor red 610 (Orange Channel)**	**Cy5/ Quasar 670 (Red Channel)**	
≤40	N/A	N/A	≤40*	Positive to SARS-CoV-2 RNA
N/A	≤40	N/A	≤40*	Positive to Flu A/B RNA
N/A	N/A	≤40	≤40*	Positive to RSV A/B RNA
≤40	≤40	≤40	≤40*	Positive to SARS-CoV-2, Flu A/B and RSV A/B RNA
≤40	≤40	N/A	≤40*	Positive to SARS-CoV-2 and Flu A/B RNA
≤40	N/A	≤40	≤40*	Positive to SARS-CoV-2 and RSV A/B RNA
N/A	≤40	≤40	≤40*	Positive to Flu A/B and RSV A/B RNA
N/A	N/A	N/A	≤40	Negative to SARS-CoV-2, Flu A/B and RSV A/B RNA
N/A	N/A	N/A	>40 o N/A	Invalid result (repeat the test starting from the extraction step). If the same result is obtained again, a new sampling must be required.

* The amplification of the RNaseP endogenous control may be absent when in presence of positive samples in which the viral target genes are preferentially amplified.

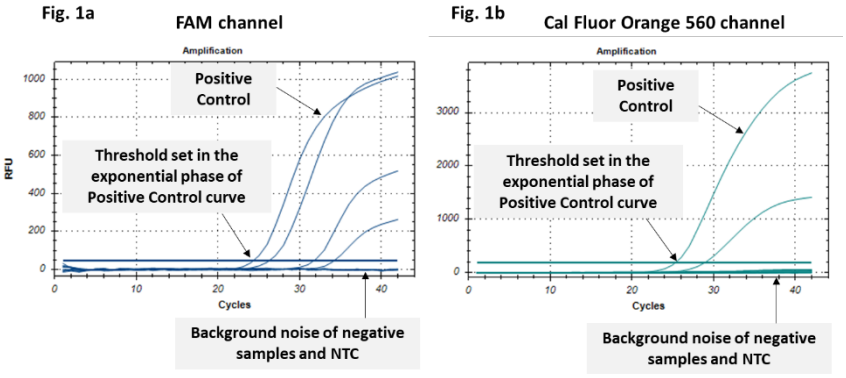
9.6. TROUBLESHOOTING

OBSERVATION	PROBABLE CAUSES	SOLUTION
No amplification signal for positive control and samples	Instrument not correctly set up	Verify the thermal protocol settings and repeat the test with the right settings
	Wrong reagent conservation or use of the reagents beyond the expiration date.	Verify the storage conditions and use a new kit if necessary
No amplification signal or very low signal of endogenous control (RNase P)	Pathogen's nucleic acid in high concentration	If in presence of a pathogen amplification signal but not the internal control signal probably the Internal Control is inhibited by the high viral titer.
	Incorrect sample collection	Repeat the sample collection and repeat the test from the RNA extraction step.
	Problem in the extraction process	Repeat the test starting from the extraction step
Presumed false positive or target signal in the PCR Negative Control	Contamination	Decontaminate all the surfaces and the work areas with sodium hypochlorite. Use filter tips. Repeat the entire procedure starting from the extraction step.
Presumed false negative or signal absence for the positive control	Wrong sample collection	Verify the sample collection method.
	Wrong RNA sample conservation	Collect the sample again and repeat the entire procedure starting from the extraction step following the recommendations.
	Incorrect nucleic acid extraction	Verify the extraction protocol and repeat the entire procedure
	Presence of PCR inhibitors	Dilute the sample
	Wrong nucleic acid addition in the amplification tube/well	Verify the correct sample addition to the corresponding amplification tube/well basing on the extraction method used.
	Incorrect reaction mix preparation	Confirm the addition of all the components to the reaction mix. All the reagents should be well mixed and centrifuged before the use.
Presence of abnormal fluorescence signals	Amplification reaction not centrifuged	Repeat the amplification reaction after spinning the plate and/or the tubes before starting.
	Sample evaporation in the thermal cycler due to erroneous reaction tubes' sealing	Repeat the amplification reaction making sure to properly seal the plate or the tubes before starting.
	Not suitable thermal cycler plastics	Contact Diatheva and repeat the test with the suggested plastics
	Presence of bubbles in the reaction tube/well	Repeat the amplification reaction after spinning the plate and /or the tubes, verify the absence of bubbles before starting the reaction.

APPENDIX A - CFX96 Analysis parameters

In the Setting topic select "baseline setting" and set up "baseline substracted curve fit". To set the threshold, manually move the line in the exponential phase of the Positive Control fluorescence curves observed in the amplification graph in **linear phase**. An example of analysis for the SARS-CoV-2 target, also valid for RSV A / B and RNase P, is shown in Figure 1a while an example of analysis for the Flu A / B target is shown in Figure 1b. If in some samples the fluorescence signal has an anomalous trend or artifacts appear, it is possible to improve the trend of the curves by selecting "settings", "Baseline threshold": select the samples with anomalous trend and set the value 20 in the respective "baseline end " box

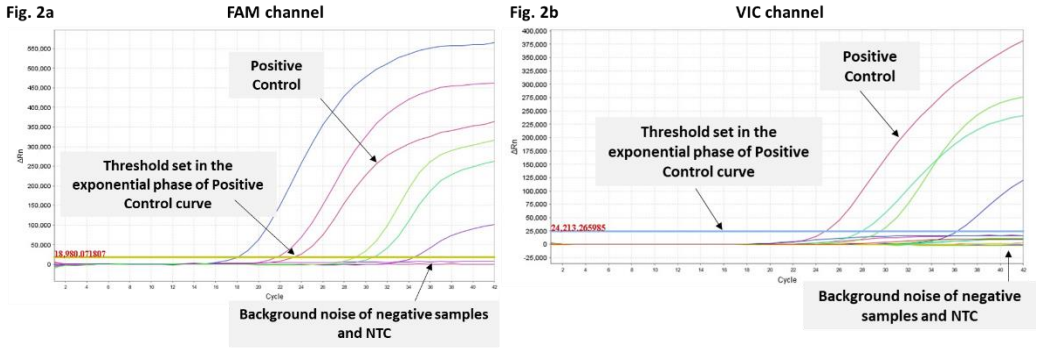
Fig 1: Parameter setting and data analysis in linear phase on CFX96 for the FAM - SARS-CoV-2 channel (Fig.1a) and for the Cal Fluor Orange 560 - Flu A / B channel (Fig.1b)



APPENDIX B - ABI 7500 Analysis parameters

For analysis, in the screen "Amplification plot" and function "Options", keep "Auto baseline" selected and deselect "Auto" for the threshold field. To set the threshold, manually move the line in the exponential phase of the Positive Control fluorescence curves observed in the amplification graph in **linear phase**. An example of analysis for the SARS-CoV-2 target, also valid for RSV A / B and RNase P, is shown in Figure 2a while an example of analysis for the Flu A / B target is shown in Figure 2b.

Fig 2: Parameter setting and data analysis in linear phase on ABI 7500 for the FAM - SARS-CoV-2 channel (Fig.2a) and for the Cal Fluor Orange 560 - Flu A / B channel (Fig.2a)

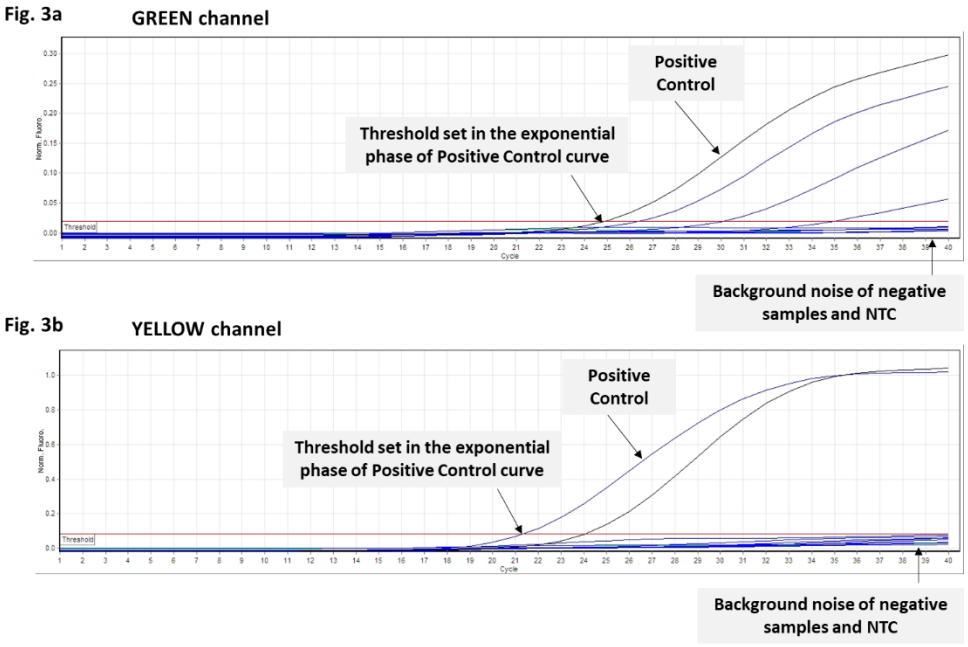


APPENDIX C – Rotor gene Q 5-plex Analysis parameters

For each fluorophore proceed with the analysis as follows:

Select the "Dinamic Tube" function. Select the "Ignore First" function by entering the value 15 in the "Cycles" box. To set the threshold, manually move the line in the exponential phase of the Positive Control fluorescence curves observed in the amplification graph in **linear phase**. An example of analysis for the SARS-CoV-2 target, also valid for RSV A / B and RNase P, is shown in Figure 3a while an example of analysis for the Flu A / B target is shown in Figure 3b.

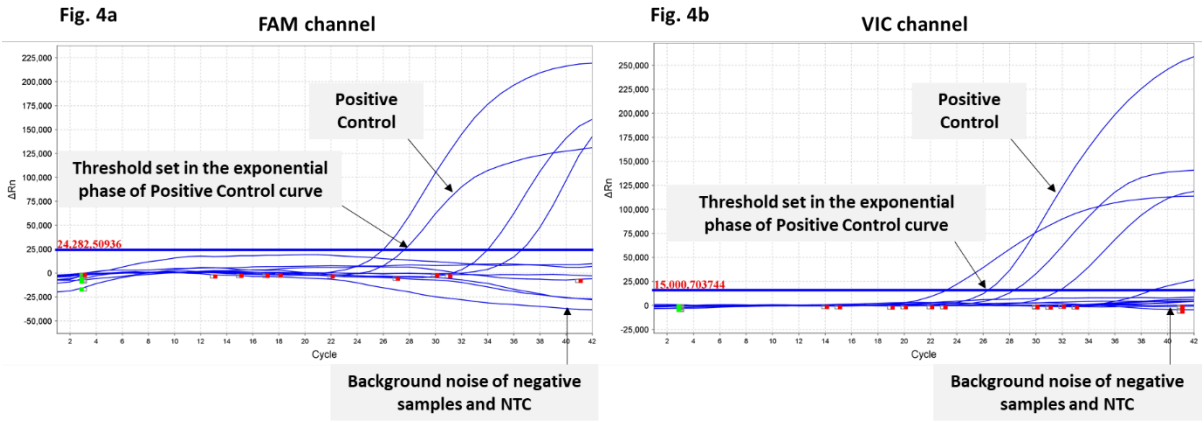
Fig 3: Parameter setting and data analysis in linear phase on Rotor gene Q 5-plex for the GREEN - SARS-CoV-2 channel (Fig.3a) and for the YELLOW - Flu A / B channel (Fig.3a)



APPENDIX D – Quant Studio 5 Analysis parameters

For the analysis, on the "Results" screen, select "Amplification Plot" from the drop-down menu, keep "Auto baseline" selected and deselect "Auto" for the threshold field. To set the threshold, manually move the line in the exponential phase of the Positive Control fluorescence curves observed in the amplification graph in **linear phase**. An example of analysis for the SARS-CoV-2 target, also valid for RNase P, is shown in Figure 4a while an example of analysis for the Flu A / B target, also valid for RNase P, is shown in Figure 4b.

Fig 4: Parameter setting and data analysis in linear phase on Quant Studio 5 for the FAM - SARS-CoV-2 channel (Fig.4a) and for the VIC - Flu A / B channel



(Fig.4b)

APPENDIX E – KIT PERFORMANCES

ANALYTICAL SPECIFICITY

The analytical specificity of the *COVID-FLU-RSV All-in-One RT PCR kit* is guaranteed by the careful and specific choice of primers and probes published in the international guidelines issued by WHO and CDC.

ANALYTICAL SENSITIVITY

The analytical sensitivity of the *COVID-FLU-RSV All-in-One RT PCR kit* was determined using the TWIST SYNTHETIC SARS-CoV-2 RNA CONTROL 2 genomic RNA (Cat. No. 102024, TWIST BIOSCIENCE) for SARS-CoV-2 target, the TWIST SYNTHETIC INFLUENZA H1N1 (2009) RNA CONTROL (Cat. No. 103001, TWIST BIOSCIENCE) for the Influenza A, the TWIST SYNTHETIC INFLUENZA B RNA CONTROL BSL-1 (Cat. No. 103003, TWIST BIOSCIENCE) for Influenza B target and the AMPLIRUN RESPIRATORY SYNCYTIAL VIRUS (subtype A) RNA CONTROL (Cat. No. MBC041, Vircell) for the RSV target. The RNA was subjected to serial dilutions and the results were analyzed by Probit analysis ($p = 0.005$, 95% CI) on the ABI7500 instrument (Applied Biosystems). The LOD determined for SARS-CoV-2 target corresponds to 3.83 copies/PCR, for Influenza A it is 16.59 copies/PCR, for Influenza B it is 2.39 copies/PCR and for RSV it is 1.28 copies/PCR.

REPEATABILITY

The repeatability of the test (*intra-assay* variability) was assessed by analyzing replicates of 5 different clinical samples in one run on CFX96 instrument (Biorad). Tested samples include a high SARS-CoV-2 positive sample, a medium SARS-CoV-2 positive sample, a low SARS-CoV-2 positive sample, a positive Influenza A/B sample and a positive RSV sample. The percentage coefficient of variation (CV%) for the COVID-19, FluA/B and RSV A/B target is shown in Table A and for all target viruses it is always <1.0:

Table A: mean Ct and CV% of SARS-CoV-2, FluA/B and RSV A/B positive samples

	Mean Ct value (CV%)				
	High positive SARS-CoV-2 sample	**Medium positive SARS-CoV-2 sample**	**Low positive SARS-CoV-2 sample**	**Positive FluA/B sample**	**Positive RSV A/B sample**
SARS-CoV-2	26.24 (0.27)	31.55 (0.90)	34.31 (0.31)	-	-
Flu A/B	-	-	-	27.90 (0.63)	-
RSV A/B	-	-	-	-	32.41 (0.07)

REPRODUCIBILITY

The reproducibility of the assay *(inter-assay* variability) was evaluated by analyzing 5 clinical samples in two different runs on the ABI7500 and CFX96 instrument, respectively. Tested samples include a high SARS-CoV-2 positive sample, a medium SARS-CoV-2 positive sample, a low SARS-CoV-2 positive sample, a positive Influenza A/B sample and a positive RSV sample. The percentage coefficient of variation (CV%) for the COVID-19, FluA/B e RSV A/B target is shown in Table B and for all target viruses it is always ≤0.8:

Table B: mean Ct and CV% of SARS-CoV-2, FluA/B and RSV A/B positive sample

	Mean Ct value (CV%)				
	High positive SARS-CoV-2 sample	**Medium positive SARS-CoV-2 sample**	**Low positive SARS-CoV-2 sample**	**Positive FluA/B sample**	**Positive RSV A/B sample**
SARS-CoV-2	26.27 (0.16)	31.39 (0.72)	34.36 (0.60)	-	-
Flu A/B	-	-	-	27.84 (0.33)	-
RSV A/B	-	-	-	-	32.44 (0.22)

ROBUSTNESS

The robustness of the method was evaluated by analyzing the impact of different operators and different thermal cyclers (Table C).

Table C: Positive samples diagnosed in different thermal cyclers by different operators

	Positivi diagnosticati/Totale positivi	**% diagnosticati**
CFX96 (Operator 1)	34/34	100
ABI 7500 (Operator 2)	42/42	100
QuantStudio 5 (Operator 3)	30/30	100
RGQ 5-plex (Operator 2)	26/26	100

For all instruments and all operators, 100% agreement was obtained with respect to the diagnosis of the samples.

CLINICAL EVALUATIONS

SARS-CoV-2

Clinical specificity was evaluated by testing 90 SARS-CoV-2 negative samples and clinical sensitivity was assessed by testing 74 SARS-CoV-2 positive samples. All samples analyzed were diagnosed by a CE-IVD marked RT-PCR method. RNAs were extracted using the extraction kits listed in the section 5 "Required material not supplied". Results are summarized in Table D.

Per tutti gli strumenti e tutti gli operatori si è ottenuta una concordanza del 100% rispetto alla diagnosi dei campioni.

Table D: Specificity, sensitivity and Kappa of Cohen value for SARS-CoV-2

SARS-CoV-2		**CE-IVD Competitor**		
		Positives	**Negatives**	**Total**
COVID-FLU-RSV All-in-One RT PCR	**Positives**	74	0	74
	Negatives	0	90	90
	Total	74	90	164

- Clinical sensitivity: 100%
- Clinical specificity: 100%
- Kappa of Cohen value: 1.00 (perfect concordance)

Influenza A/B

Clinical specificity was evaluated by testing 117 Flu A/B negative samples. Clinical sensitivity was assessed by testing 22 Flu A/B positive samples. All samples analyzed were diagnosed by a CE-IVD marked reference RT-PCR method. Results are summarized in Table E.

Table E: Specificity, sensitivity and Kappa of Cohen value for Flu A/B

FluA/B		**CDC reference method**		
		Positives	**Negatives**	**Total**
COVID-FLU-RSV All-in-One RT PCR	**Positives**	22	0	22
	Negatives	0	117	117
	Total	22	117	139

- Clinical sensitivity: 100%
- Clinical specificity: 100%
- Kappa of Cohen value: 1.00 (perfect concordance)

RSV A/B

Clinical specificity was evaluated by testing 117 RSV A/B negative samples. Clinical sensitivity was assessed by testing 22 RSV A/B positive samples. All samples analyzed were diagnosed by a CE-IVD marked reference RT-PCR method. Results are summarized in Table F.

Table F: Specificity, sensitivity and Kappa of Cohen value for RSV A/B

RSV A/B		**CDC reference method**		
		Positives	**Negatives**	**Total**
COVID-FLU-RSV All-in-One RT PCR	**Positives**	22	0	22
	Negatives	0	117	117
	Total	22	117	139

- Clinical sensitivity: 100%
- Clinical specificity: 100%
- Kappa of Cohen value: 1.00 (perfect concordance)

Clinical validation was carried out in collaboration with the regional reference center for virological surveillance of influenza and COVID-19, Department of Biomedical Sciences for Health, Università degli Studi of Milan.