Via Sant'Anna 131-135
61030 Cartoceto PU (IT)
Telephone +39 (0)721 830605
FAX +39 (0)721837154
e-mail: info@diatheva.com
www.diatheva.com

COVID-FLU-RSV RT PCR Detection kit

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INTENDED USE

COVID-FLU-RSV RT PCR Detection kit is a multiplex real-time RT-PCR assay one-step for the qualitative detection of RNA from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Influenza A (Flu A) virus, Influenza B virus (Flu B) and Respiratory syncytial virus A/B (RSV A/B) in naso-pharingeal and saliva samples. The test is intended for differential diagnosis of infections by SARS-CoV-2, Influenza A, Influenza B and RSV in combination with other clinical and epidemiological data of patients.
Positive results are indicative for the presence of RNA from SARS-CoV-2, FluA, FluB and RSV. 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, FluA, FluB and RSV A/B and cannot be used as unique information for the clinical management of the patients. Negative results must be combined with clinical observations, patient 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.

INTRODUCTION

The use of tests that 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 SARS-CoV-2, the ability to use multiplex-type assays will be essential in order to implement effective disease control as well as clinical treatment.

PRINCIPIO DEL SAGGIO

COVID-FLU-RSV RT PCR Detection kit is a RT-PCR system that allows the RNA amplification and differentiation of SARS, human Influenza A, B and respiratory syncytial viruses A/B.
Starting from the extracted RNA, two reverse transcription and amplification reactions are carried out:

- one specific for SARS-CoV-2 through the simultaneous amplification of 3 specific viral genes (ORF1b / RdRp, S and N) and the target **RNase P** as an internal positive control (IC) to monitor that the sample is of acceptable quality, the RNA extraction process and the presence of PCR inhibitors.
- one specific for Flu A, Flu B and RSV and of the target **RNase P** as an internal positive control (IC) as noted above.

KIT CONTENT

The kit provides all the reagents necessary for the analysis. Negative and Positive PCR control are included for both multiplex COVID-19 and FLU-RSV.

Table 1: Kit content

Reagent	No vial x Volume	Cap color
Mix 1	2X 690 µL	Green
Mix 2	2 X 86 µL	Orange
Mix Primer/Probe COVID-19	2 X 880 µL	Blue
Mix Primer/Probe FLU-RSV	2 X 880 µL	Yellow
PCR Negative Control	1 X 100 µL	Clear
PCR Positive Control COVID-19	1 X 50 µL	Red
PCR Positive Control FLU-RSV	1 X 50 µL	Violet

REQUIRED MATERIALS NOT SUPPLIED

- Disposable powder-free gloves
- RNA isolation kit
- Pipettes (adjustable)
- Sterile pipette tips with aerosol-preventive filters
- Vortex mixer
- Bench Microcentrifuge

- Real-time PCR instrument
- Consumables for real-time PCR instruments, clear plates, clear 8-well tubes, optical sealing foils, optical 8 caps tubes
- 1.5mL tubes
- Laboratory freezers -30°C to -10°C/-70°C

The kit is validated to be used with the following extraction methods and thermocycler instruments:

Thermalcyclers
- CFX96 Biorad
- ABI7500 Applied Biosystems
- Rotor gene Q 5-plex

Extraction methods
- RNeasy Mini kit (Qiagen)
- High Pure Viral RNA/DNA Kit (Magen)
- MGISP-960 platform using the Virus DNA/RNA Extraction kit
- KingFisher™ Flex Purification System using MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit
- Purification system using OMNIA LH75 with NucleoMag Pathogen (Macherey-Nagel)
- Total RNA Purification kit (Norgen)

For use with other Real-Time PCR instruments and extraction methods, please contact Diatheva

ASSAY LIMITATIONS

- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper sample collection, transfer, storage and processing may cause erroneous test results
- The kit uses purified RNA as a sample for the analysis. The quality of the RNA recovered from biological samples is essential for the quality of results generated with this kit.
- The kit has been designed and validated for the use with the real time PCR instrument CFX96 (Biorad)
- Diatheva can't respond of results obtained using instruments or accessorize other than recommended in the user manual
- 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 polymorphisms in the target regions in which the sequences hybridize (primers and probes) could compromise the detection of the targets
  - Failure to follow instruction for use
- False positive results may arise from:
  - Cross contamination during specimen collection handling or preparation
  - RNA contamination during product handling

SHIPPING AND STORAGE

Shipping in dry ice. Upon arrival, store at -20°C. If stored at the recommended temperature all reagents are stable until the expiration date.

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;
- Positive results are indicative of the presence of SARS-CoV-2, FLUA FLUB and RSV RNA
- The quality of the sample preparation may influence the quality of qPCR test.

INSTRUCTION FOR USE

1. Sample collection and preparation

For collection methods refer to specimen collection devices manufacturer instructions. For the collection of upper and lower respiratory samples the use of a 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.

The samples should be extracted according to the corresponding requirements and procedures of 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 in the table 6. For additional extraction methods not listed in the table please contact Diatheva.
The extracted RNA sample can be stored at below -70°C, avoiding repeated freeze-thaw.

2. Set-up real time PCR instrument

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

Table 2: Thermal profile setting

Phase	Temperature and Time	Cycles
cDNA Synthesis	48°C 10 min	1 X
Initial denaturation	95°C 10 min	1 X
Denaturatution	95°C 15 sec	45 X**
Annealing-extension*	58°C 60 sec	

*Fluorescence is detected during **annealing-extension** step on channel specified in the following table:

** perform 40 cycles using the instrument Rotor gene Q

Table 3: Fluorescence channels setting

Multiplex	Fluorescence channels			
	FAM	VIC/Cal FluorO range 560	Cal Fluor red 610/ROX	CY5/Quasar 670
Target Multiplex COVID	ORF1b/RdRp gene	S gene	N gene	RNaseP
Target Multiplex FLU-RSV	M1 gene (Flu A)	NS2 gene (FluB)	M gene (RSV)	RNaseP

- The final reaction volume is **25 µL or 27 µL (to insert final reaction volume consider the RNA sample volume to be added according to the table 6)**
- **For the instruments that require select "none" in the function "select dye to use as passive reference"**
- **3. Reactions set-up** For the Rotor gene Q Instrument, place the NTC of the COVID-19 MIX in position 1 and set the "Auto-gain optimization setup" function, select the function "Perform optimization before 1st acquisition". Finally select Optimize acquiring by selecting all the fluorescence reading channels shown in the table 3 for each target.

The setup of the multiplex reactions for COVID-19 and FLU-RSV requires the preparation of two separate mastermixes therefore each sample to be tested, PCR Negative Control and the respective PCR Positive Control must be tested with both mastermixes. The preparation of Mastermix COVID-19 and Mastermix FLU-RSV must be performed according to the following instructions:

Note: Keep both COVID-19 Primer / Probe Mix and FLU-RSV Primer / Probe Mix protected from light

- Thaw the components. Vortex Mix 1, Mix Primer / Probe COVID-19 and Mix Primer / Probe FLU-RSV for 15 sec and centrifuge briefly. Vortex Mix 2 for 2 sec and centrifuge briefly.
- In two separate sterile 1.5 ml tubes, prepare the amplification mastermixes required for each sample to be tested plus a Negative Control and a Positive Control, following the scheme shown in the following tables:

Table 4: COVID-19 Mastermix preparation

Reagent	Reagent Volume for 1 reaction*
Mix 1**	6.25 µL
Mix 2**	0.78 µL
Mix Primer/Probe COVID-19	15.97 µL
Total Volume	23 µL

Table 5: FLU-RSV Mastermix preparation

Reagent	Reagent Volume for 1 reaction*
Mix 1**	6.25 µL
Mix 2**	0.78 µL
Mix Primer/Probe FLU-RSV	15.97 µL
Total volume	23 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 to be tested considering the NTC and Positive Control.
** Reagents Mix 1 and Mix 2 contains component provided by Solis BioDyne

- Vortex the tubes containing the master mixes for 10” and centrifuge briefly
- Aliquot **23 µL** of each Master mix in the PCR tubes or in the wells of the plate prepared for the experiment
- In a separate area, add the PCR Negative Control, the RNA sample to be analyzed, the Positive Control COVID-19 and the Positive Control FLU-RSV in the corresponding wells in which the respective master mix has been previously aliquoted following the instructions given in the table below:

Table 6: RNA samples loading

Extraction type*	RNA sample Volume	PCR Negative Control Volume	PCR Positive Control Volume
• RNeasy Mini kit (Qiagen) • High Pure Viral RNA/DNA Kit (Magen) • KingFisher™ Flex Purification System using MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit • Purification system using OMNIA LH75 with NucleoMag Pathogen (Macherey-Nagel) • Total RNA Purification kit (Norgen)	**4 µL**		
MGISP-960 platform using the Virus DNA/RNA Extraction kit	**2 µL**		
WARNING: In the case of different extraction kits than those recommended, it is the user's responsibility to verify through the use of standard samples (eg. VEQ) that this extraction does not lead to a reduction in the performance of the analysis system			

- Seal hermetically the PCR plate and load into the real-time PCR instrument, following the manufacturer’s instructions

Note: check that the reagents are at the bottom of each well, if not, centrifuge at 800 x g for 1 minute.

4. Analysis 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.

Using the instruments CFX96, Rotorgene Q 5-plex, ABI 7500, QuantStudio5 refer respectively to **appendices A, B, C, D** for the set-up of the analysis parameters.

The use of the COVID-19 positive controls, FLU-RSV positive control and negative control in each run validates the reaction by verifying the presence of the signals as reported below in the positive control wells and the absence of signal in the negative control well. Before interpreting the sample results it is necessary to verify the execution of the PCR.
Proceed with checking the controls based on the following table:

Table 7: Reaction controls Check

Control	Fluorescence Channel			
	FAM	**VIC/Cal Fluor Orange 560**	**Cal Fluor red 610 /ROX**	**Cy5/ Quasar 670**
PCR Positive Control COVID-19	Positive (Orf1b/RDRP)	Positive (S)	Positive (N)	Positive (RNaseP)
PCR Positive Control FLU-RSV	Positive (FluA)	Positive (FluB)	Positive (RSV)	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 before to proceed.

Table 8: Analysis of results for the COVID-19 reaction multiplex

Amplification				Interpretation of results
ORF1b/ RdRp FAM	**S VIC/Cal Fluor Orange 560**	**N Cal Fluor red 610 /ROX**	**RNaseP Cy5/ quasar 670**	
Positive	Positive	Positive	≤40*	Positive for SARS-CoV-2 RNA
Positive	Positive	N/A	≤40*	Positive for SARS-CoV-2 RNA one or more negative targets may be indicative of: - Concentration of analyte below or close to the LOD - Presence of mutations in the target region - Other factors
Positive	N/A	N/A	≤40*	
N/A	Positive	Positive	≤40*	
N/A	Positive	N/A	≤40*	
N/A	N/A	Positive	≤40*	
N/A	N/A	N/A	≤40*	Negative for the presence of SARS-CoV-2 RNA
N/A	N/A	N/A	>40 o N/A	Invalid result (repeat test). If the same result is obtained again, a new sampling must be requested.

Table 9: Analysis of results for the FLU-RSV reaction multiplex

Amplification				Interpretation of results
FLU A FAM	**FLU B VIC/Cal Fluor Orange 560**	**RSV Cal Fluor red 610 /ROX**	**RNaseP Cy5/ quasar 670**	
Positive	N/A	N/A	≤40*	Positive for FLU A RNA
N/A	Positive	N/A	≤40*	Positive for FLU B RNA
N/A	N/A	Positive	≤40*	Positive for RSV RNA
Positive	Positive	N/A	≤40*	Positive for FLU A and FLU B RNA
N/A	Positive	Positive	≤40*	Positive for FLU B and RSV RNA
Positive	N/A	Positive	≤40*	Positive for FLU A and RSV RNA
N/A	N/A	N/A	≤40	Negative for all targets FLU A FLU B and RSV
N/A	N/A	N/A	>40 o N/A	Invalid result (repeat test). If the same result is obtained again, a new sampling must be requeste

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

Appendix A-CFX96 Analysis parameters

For each fluorophore select "settings", "baseline threshold" and "single threshold-user defined", entering the following values and click OK:

Target/Fluorophore	Threshold value
RDRP/FAM	80
S/Cal Orange 560	30
N/Cal red 610	40
RnaseP/Quasar 670	30

ATTENTION: for a correct interpretation of the results, pay attention to the amplification curves.

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

Appendix-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 10 in the "Cycles" box.
To set the threshold, manually move the line in the exponential phase of the fluorescence curves (Fig. 1).

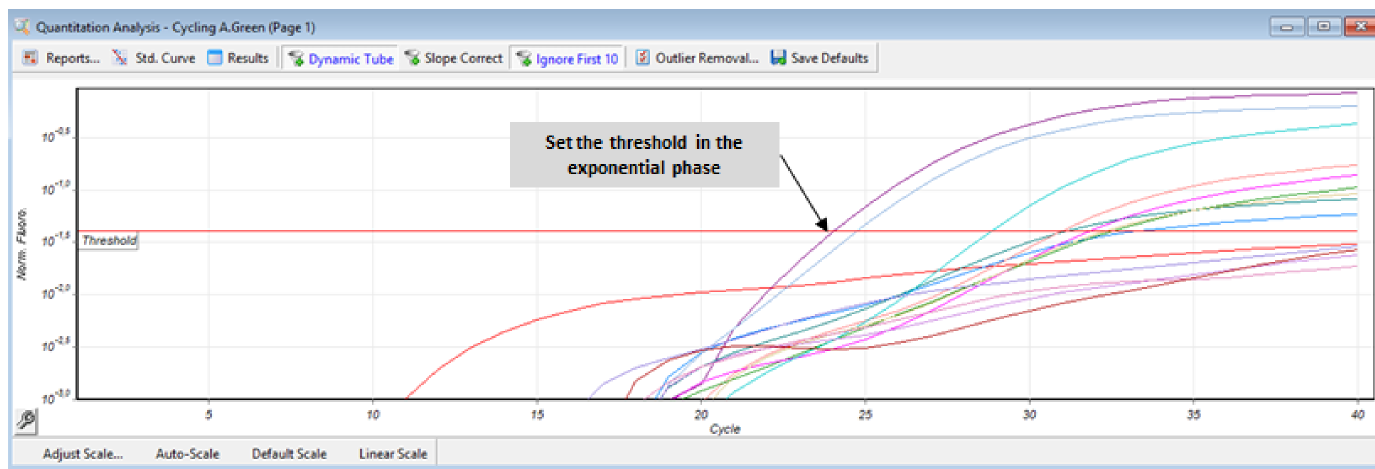

Fig 1: Parameter setting and data analysis on Rotor gene Q 5-plex

Appendix C-ABI 7500 Analysis parameters

For analysis, in the screen "Amplification plot" and function "Options", deselect "Auto Baseline" and "Auto" respectively at the threshold.
To set the threshold, manually move the line in the exponential phase of the fluorescence curves (Fig. 2).

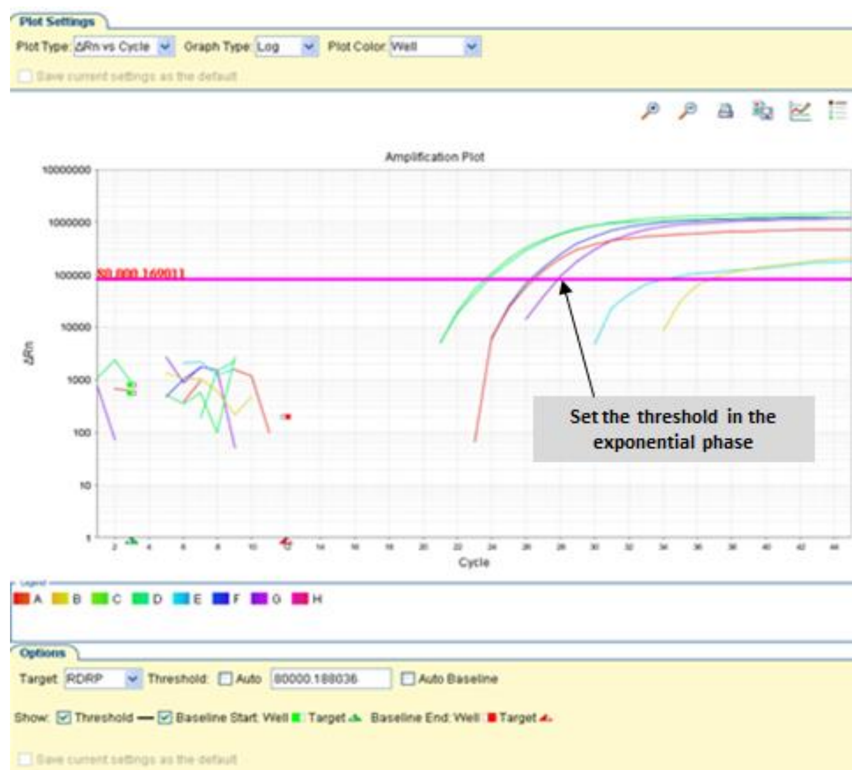

Fig 2: Parameter setting and data analysis on ABI 7500

APPENDIX D - Quant Studio 5 Analysis parameters

For the analysis, on the "Results" screen, select "Amplification Plot" from the drop-down menu. Select one target at a time. Click the "Show Plot Settings" symbol and deselect "Auto Baseline" and "Auto" respectively at the threshold. To set the threshold, manually move the line in the exponential phase of the fluorescence curves (Fig.3).

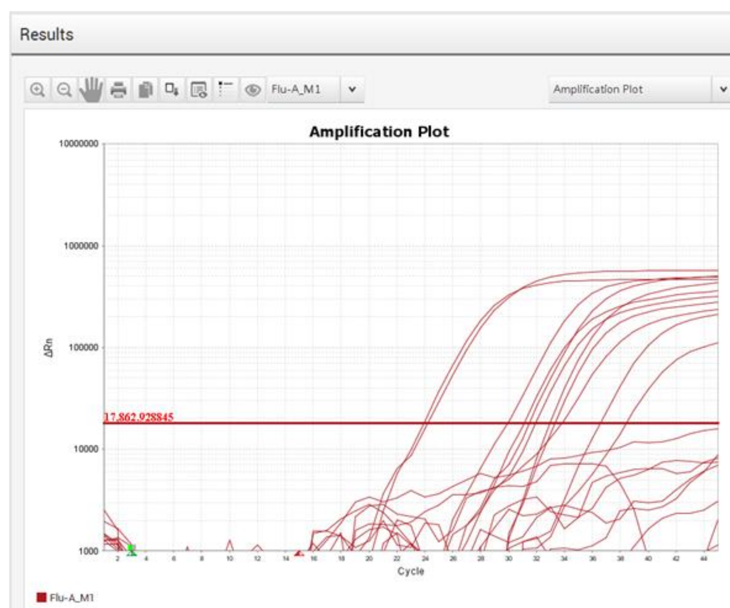

Fig 3: Parameter setting and data analysis on Quant Studio 5

PERFORMANCE VALIDATION

ANALYTICAL SPECIFICITY

The analytical specificity of the "COVID-FLU-RSV RT PCR Detection 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 Limit of Detection (LoD) was determined by testing serial dilutions of a synthetic construct containing the three COVID target regions and of a construct containing the FLU / RSV target regions. Results were analyzed by Probit statistic (p = 0.005, 95% CI) on the CFX96 and ABI 7500 instruments.

Table A: SARS CoV-2 LoD

Virus	Target gene	LOD (copies/reaction)
SARS-CoV-2	*RdRp/ORF1b*	*4.07*
	S	*5.01*
	N	*11.7*

Table B: FluA, FluB e RSV A/B LoD

Virus	Target gene	LOD (copies/reaction)
FluA	*M1*	*12.90*
FluB	*NS2*	*14.45*
RSV A/B	*M*	*10.70*

REPEATABILITY

Mix COVID-19 (SARS-CoV-2)

The repeatability of the test (*intra-assay* variability) was assessed by analyzing replicates of three different clinical samples in one run on CFX96 instrument (Biorad). Tested samples include an high COVID-19 positive sample, a medium COVID-19 positive sample and a low COVID-19 positive sample. The percentage coefficient of variation (CV%) for the SARS-CoV-2 target genes is shown in Table C and for all virus targets it is always <2:

Table C: mean Ct and CV% of SARS-CoV-2 positive samples

Mix COVID-19	Mean Ct value (CV%)		
	High positive sample	Medium positive sample	Low positive sample
ORF1b/RdRp	19.37 (0.37)	26.95 (1.50)	31.00 (0.43)
S	18.49 (0.04)	25.75 (0.74)	30.27 (0.47)
N	19.76 (0.47)	26.85 (0.97)	31.85 (0.80)

Mix FLU-RSV (FluA, FluB, RSV A/B)

The repeatability of the test was assessed by analyzing replicates of three different clinical samples in one run on CFX96 instrument. Analyzed samples include an Influenza A positive sample, an Influenza B positive sample and a RSV positive sample. CV% for FluA, FluB and RSV A / B targets is shown in Table D and for all viruses it is always <1:

Table D: mean Ct and CV% of FluA, FluB and RSV positive samples

Mix FLU-RSV	Mean Ct value (CV%)		
	FluA positive sample	FluB positive sample	RSV A/B positive sample
FluA	31.72 (0.25)	-	-
FluB	-	31.02 (0.59)	-
RSV A/B	-	-	38.51 (0.94)

REPRODUCIBILITY

Mix COVID-19 (SARS-CoV-2)

The reproducibility of the assay (inter-assay variability) was evaluated by analyzing three clinical samples in two different runs on the CFX96 and ABI 7500 instruments. Tested samples include an high COVID-19 positive sample, a medium COVID-19 positive sample and a low COVID-19 positive sample. The CV% for SARS-CoV-2 target genes is shown in Table E and for all virus targets it is always ≤3:

Table E: mean Ct and CV% of SARS-CoV-2 positive samples

Mix COVID-19	Mean Ct value (CV%)		
	High positive sample	Medium positive sample	Low positive sample
ORF1b/RdRp	18.84 (2.82)	31.73 (2.05)	33.12 (2.52)
S	17.25 (1.19)	31.31 (3.00)	32.61 (0.15)
N	17.46 (1.34)	33.32 (2.80)	34.73 (1.06)

Mix FLU-RSV (FluA, FluB, RSV A/B)

The reproducibility of the test was assessed by analyzing three different clinical samples in two different runs on CFX96 and ABI 7500. Analyzed samples include an Influenza A positive sample, an Influenza B positive sample and a RSV positive sample. CV% for FluA, FluB and RSV A / B targets is shown in Table F and for all viruses it is always <2:

Table F: mean Ct and CV% of FluA, FluB and RSV positive samples

Mix FLU-RSV	Mean Ct value (CV%)		
	FluA positive sample	FluB positive sample	RSV A/B positive sample
FluA	31.11 (0.09)	-	-
FluB	-	30.76 (1.10)	-
RSV A/B	-	-	37.06 (0.04)

ROBUSTNESS

Mix COVID-19 (SARS-CoV-2)

The robustness of the method was evaluated by analyzing the impact of different extraction methods (Table G), different operators and different thermal cyclers (Table H).

Table G: Ct and CV% value of samples extracted with manual method (Magen) and automatic method (MGI)

Mix COVID-19	High positive sample			Medium positive sample			Low positive sample		
	MGI* (Ct)	Magen** (Ct)	CV(%)	MGI* (Ct)	Magen** (Ct)	CV(%)	MGI* (Ct)	Magen** (Ct)	CV(%)
ORF1b/RdRp	18.37	19.47	4.11	29.73	31.13	3.25	33.88	33.41	0.99
S	17.07	18.03	3.87	29.11	30.14	2.46	33.59	32.94	1.38
N	17.91	18.87	3.69	31.09	31.81	1.62	35.43	35.17	0.52
RNse P	27.22	29.59	5.90	28.42	29.03	1.5	30.6	30.49	0.25

* High Pure Viral RNA/DNA Kit (Magen)
** MGISP-960 platform using Virus DNA/RNA Extraction kit (MGI)

Obtained results show a 100% agreement in the diagnosis of all targets for all samples extracted with the two different extraction methods. CV% for the SARS-CoV-2 target genes, calculated from the Ct values obtained with the two extraction methods, is always ≤6:

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

Mix COVID-19	Diagnosed positive samples/Total positive samples	% Diagnosed samples
CFX96 Operator 1	68/68	100
ABI 7500 Operator 2	23/23	100
RGQ 5-plex Operator 2	10/10	100

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

Mix FLU-RSV (FluA, FluB, RSV A/B)

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

Table I: Positive samples diagnosed in different cyclers by different operators

Mix COVID-19	Diagnosed positive samples/Total positive samples	% Diagnosed samples
CFX96 Operator 1	14/14	100
Quant Studio 5 Operator 2	134/134	100

CLINICAL EVALUATIONS

Mix COVID-19 (SARS-CoV-2)

- Upper and lower respiratory samples

Clinical specificity was evaluated by testing 19 upper and lower respiratory 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 Table 6. Results are summarized in Table L.

Table L: SARS-CoV-2 specificity and sensitivity

SARS-CoV-2		CE-IVD Approved Comparator		
		Positive	**Negative**	**Total**
COVID-FLU-RSV RT PCR Detection kit	**Positive**	74	0	74
	Negative	0	19	19
	Total	74	19	93

- Clinical specificity: 100%
- Clinical sensitivity: 100%

- Saliva samples

Clinical specificity was evaluated by testing 5 saliva SARS-CoV-2 negative samples and clinical sensitivity was assessed by testing 10 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 Table 6. Results are summarized in Table M.

Table M: SARS-CoV-2 specificity and sensitivity

SARS-CoV-2		CE-IVD Approved Comparator		
		Positive	**Negative**	**Total**
COVID-FLU-RSV RT PCR Detection kit	**Positive**	7	3	10
	Negative	0	5	5
	Total	7	8	15

- Clinical specificity: 70%
- Clinical sensitivity: 100%

Mix FLU-RSV (FluA, FluB, RSV A/B)

Clinical specificity was evaluated by testing 19 FluA negative, 19 FluB negative and 19 RSV negative samples. Clinical sensitivity was assessed by testing 50 samples for FluA, FluB and 37 RSV positive samples. All analyzed samples were diagnosed by a Reference RT-PCR method. Results are summarized in Tables N, O, P.

Table N: FluA specificity and sensitivity

FluA		CE-IVD Approved Comparator		
		Positive	**Negative**	**Total**
COVID-FLU-RSV RT PCR Detection kit	**Positive**	50	0	50
	Negative	0	19	19
	Total	50	19	69

- Clinical specificity: 100%
- Clinical sensitivity: 100%

Table O: FluB specificity and sensitivity

FluB		CE-IVD Approved Comparator		
		Positive	**Negative**	**Total**
COVID-FLU-RSV RT PCR Detection kit	**Positive**	49	0	49
	Negative	0	19	19
	Total	49	19	68

- Clinical specificity: 100%
- Clinical sensitivity: 100%

Table P: RSV specificity and sensitivity

RSV A/B		CE-IVD Approved Comparator		
		Positive	**Negative**	**Total**
COVID-FLU-RSV RT PCR Detection kit	**Positive**	37	0	37
	Negative	0	19	19
	Total	37	19	56

- Clinical specificity: 100%
- Clinical sensitivity: 100%