VIASURE

Real Time PCR Detection Kits

***SARS-CoV-2, Flu & RSV* Technical report**

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1 AIM

The aim of this document is to provide a brief description of the trials performed on VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit in order to evaluate the following performance characteristics: analytical sensitivity, precision (intra- and inter-assays and inter-batches), linearity, analytical specificity and reactivity, clinical evaluation studies, interferences and inhibitors of PCR and stability assays in order to demonstrate that VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit is suitable for its intended use and meets the according pre-determined criteria. The protocol of the validation assays is described in "POC-43 Validation procedure for qPCR products".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit targets two separate and independent conserved regions of the virus nucleocapsid phosphoprotein (*N*) gene (called N1 and N2 gene targets, N1 and N2 targets or N1 and N2 regions throughout the document) of SARS-CoV-2, a conserved region of the *M1* gene for Flu A and Flu B, a conserved region of the *N* gene for RSV A and B, and the human *RNase P* gene. SARS-CoV-2 (N1/N2 targets) is amplified and detected in FAM channel, Flu A/B are amplified and detected in ROX channel, RSV A/B is amplified and detected in Cy5 channel. This assay uses a human housekeeping gene as an **endogenous Internal Control (IC)** (human *RNase P* gene), which is amplified and detected in HEX channel, VIC or JOE channel. Human housekeeping genes are involved in basic cell maintenance and, therefore, are expected to be present in all nucleated human cells and maintain relatively constant expression levels. This **endogenous Internal Control** is used to monitor the extraction process and/or discard (RT)-PCR inhibition and/or verify the correct functioning of the amplification mix.

Based on the commercial presentation and the Real Time PCR platform used, the stabilized PCR reaction mix could be placed inside different tubes or wells and could be marketed on **MULTIPLE FORMATS**:

- **8-well strips or 96-well plate in high or low profile** (open platform devices, compatibility with the most common Real Time PCR platform). The strips or plate will be closed by tear-off 8-cap strips.

- **Reaction-Mix Tube** (once the Reaction-Mix tube has been re-suspended, the reaction-mix can be added to different wells or tubes of the most common Real Time PCR platforms, as well).

- **Clear 4-well strips**, compatible with Qiagen/Corbett **Rotor-Gene® instruments**. The strip of 4 tubes will be closed by 4-cap strips.

The clinical and analytical performance studies will be mainly conducted on 8-well strip in low or high profile (open format). Since the obtained data can be extrapolated to the remaining different formats, only some representative assays will be performed to verify that meet the performance characteristics achieved (e.g. analytical sensitivity, stability studies).

Based on the similar clinical signs and symptoms common to several infections, that could be caused by different pathogens; the reaction mixes could be marketed on additional formats as **multiple wells and/or VIASURE panels**. This format consists of one strip composed for different reaction mixes (one into each well).

Therefore, multiple wells and/or VIASURE panel formats allow the simultaneous detection of most clinically relevant pathogens and/or targets which can be syndromically grouped. In particular, *SARS-CoV-2, Flu & RSV* reaction mix could be combined with other reaction mixes in multiple wells format and/or VIASURE panel format, the summarized results stated in this technical report will be extrapolated to all different formats and variants which include this reaction mix.

2 ANALYTICAL SENSITIVITY AND LINEARITY

The analytical sensitivity (linearity of the assay and tentative limit of detection or LoD) was determined by testing a series of ten-fold dilutions containing a known concentration (ranging from 10^7 to 10^1 copies per reaction) of specific and synthetic cDNA belonging to SARS-CoV-2, Flu & RSV. Every tenfold dilution was tested in triplicate. The arithmetic mean ($\bar{x}$), standard deviation (σ) and coefficient of variation (CV%) were calculated and are detailed in Tables 1, 2, 3, 4 and 5.

Examples of the amplification plots resulting from running an assay run on the Bio-Rad CFX96™ Real-Time PCR Detection System are included in the Instructions for use.

	copies/rxn							
Synthetic SARS-CoV-2 (N1/N2 regions) cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**10^1**	**0**
$\bar{x}$ (Ct)	13.72	17.16	20.44	23.88	27.18	29.92	32.27	Neg
σ	0.19	0.12	0.04	0.31	0.09	0.23	0.12	n.a.
CV%	1.39	0.72	0.17	1.31	0.33	0.76	0.36	n.a.

Table 1. Analytical sensitivity was evaluated with synthetic SARS-CoV-2 specific cDNA and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -FAM channel- (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07), **rxn** = reaction, (**Ct**) = threshold cycle, ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a tentative detection limit of ≥10 cDNA copies per reaction for SARS-CoV-2. cDNA molecules can be detected with a concentration of ≥10 copies/rxn (positive rate of ≥95%).

The qPCR efficiency was estimated at >108.2% (Slope -3.140). Linear regression showed R^2 value of 0.996.

	copies/rxn							
Synthetic Flu A cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**10^1**	**0**
$\bar{x}$ (Ct)	16.58	20.27	24.05	27.84	29.94	33.61	37.01	Neg
σ	0.09	0.05	0.10	0.25	0.07	0.50	0.32	n.a.
CV%	0.56	0.26	0.42	0.92	0.23	1.47	0.87	n.a.

Table 2. Analytical sensitivity was evaluated with synthetic Flu A cDNA and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -Cy5 channel- (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07), **rxn** = reaction, (**Ct**) = threshold cycle, ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a tentative detection limit of ≥10 cDNA copies per reaction for Flu A. cDNA molecules can be detected with a concentration of ≥10 copies/rxn (positive rate of ≥95%).

The qPCR efficiency was estimated at >95.5% (Slope -3.333). Linear regression showed R^2 value of 0.995.

	copies/rxn							
Synthetic Flu B cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**50**	**0**
$\bar{x}$ (Ct)	16.80	20.53	24.12	27.48	31.04	33.75	34.46	Neg
σ	0.04	0.23	0.11	0.25	0.92	0.62	0.32	n.a.
CV%	0.24	1.11	0.47	0.92	2.97	1.85	0.93	n.a.

Table 3. Analytical sensitivity was evaluated with synthetic Flu B cDNA and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -Cy5 channel- (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07), **rxn** = reaction, (**Ct**) = threshold cycle, ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a detection limit of ≥50 cDNA copies per reaction for Flu B. cDNA molecules can be detected with a concentration of ≥50 copies/rxn (positive rate of ≥95%).

The qPCR efficiency was estimated at >92% (Slope -3.530). Linear regression showed R^2 value of 0.978.

	copies/rxn							
Synthetic RSV A cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**10^1**	**0**
$\bar{x}$ (Ct)	15.26	18.78	22.43	26.06	28.82	32.74	35.81	Neg
σ	0.16	0.17	0.05	0.03	0.02	0.57	0.53	n.a.
CV%	1.05	0.92	0.20	0.10	0.08	1.74	1.48	n.a.

Table 4. Analytical sensitivity was evaluated with synthetic RSV A cDNA and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -Cy5 channel- (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07), **rxn** = reaction, (**Ct**) = threshold cycle, ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a tentative detection limit of ≥10 cDNA copies per reaction for RSV A. cDNA molecules can be detected with a concentration of ≥10 copies/rxn (positive rate of ≥95%).

The qPCR efficiency was estimated at >95.8% (Slope -3.428). Linear regression showed R^2 value of 0.998.

	copies/rxn							
Synthetic RSV B cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**10^1**	**0**
$\bar{x}$ (Ct)	16.90	20.43	24.81	28.00	31.01	34.79	36.84	Neg
σ	0.06	0.05	0.31	0.27	0.07	0.94	0.75	n.a.
CV%	0.33	0.22	1.25	0.95	0.22	2.69	2.04	n.a.

Table 5. Analytical sensitivity was evaluated with synthetic RSV B cDNA and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -Cy5 channel- (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07), **rxn** = reaction, (**Ct**) = threshold cycle, ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a tentative detection limit of ≥10 cDNA copies per reaction for RSV B. cDNA molecules can be detected with a concentration of ≥10 copies/rxn (positive rate of ≥95%).

The qPCR efficiency was estimated at >97.2% (Slope -3.390). Linear regression showed R^2 value of 0.990.

In conclusion, all real-time PCR assays showed an acceptable **efficiency** and **linearity**, (R^2) were >0.98 in all the target reactions tested.

The analytical sensibility of VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit was also tested on Applied Biosystems 7500 Fast Real-Time PCR System, Cobas z480 Analyzer (Roche Molecular Diagnostics) and DTprime Real-time Detection Thermal Cycler (DNA-Technology) and DNA-Technology DTlite Real-

Time PCR System (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07). The results for all targets match with the LoD which a positive rate of 95% or the follow tenfold dilution above.

Another analysis was performed to determine the LoD of SARS-CoV-2 in genome copies per reaction (genome copies/rxn). The LoD was determined by testing five times four negative clinical oropharyngeal (throat) swabs (viral transport medium, VTM- Vircell-) (total twenty times) spiked with a known concentration of frozen quantified heat-inactivated culture 2019 Novel Coronavirus, Strain:2019-nCoV/USA-WA-1/2020 (ATCC-VR-1986HK) (which were at the detection limit). The dilutions contained a known concentration of heat-inactivated culture (2.0 genome copies/µL and 1.0 genome copies/µL). The four spiked samples were extracted with MagDEA Dx SV kit (Batch n°18M010, expiry date 2022-04), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch n° CFR1XL-EXP.469C, expiry date 2022-07) in quintupled on Bio-Rad CFX96™ Real-Time PCR Detection System. The arithmetic mean ($\overline{x}$), standard deviation (σ) and coefficient of variation (CV%) were calculated and are detailed in Table 6.

	Genome copies/ rxn (VTM, Vircell)	
Negative samples spiked with quantified heat-inactivated culture 2019 Novel Coronavirus (ATCC-VR-1986HK) – N1/N2 regions	**20**	**10**
$\overline{x}$ (Ct)	33.50	34.51
σ	0.84	0.67
CV%	2.51	1.96
n	20/20	18/20

Table 6: The LoD was determined with quantified heat-inactivated culture 2019 Novel Coronavirus and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -FAM channel- (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07). **rxn** = reaction, (**Ct**) = threshold cycle, ($\overline{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, (***n***) = number of samples amplified.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a detection limit of ≥20 genome copies per reaction for SARS-CoV-2 (*N1/N2* regions) with a positive rate of ≥95%. This concentration was considered the LoD for SARS-CoV-2 (*N1/N2* regions).

The LoD of Influenza A in genome copies per reaction (genome copies/rxn) was determined by testing five times four negative clinical oropharyngeal (throat) swabs (viral transport medium, VTM- Vircell-) (total twenty times) spiked with a known concentration of frozen quantified Influenza A/PR/8/34 (H1N1) virus, purified (ATCC® VR95PQ™) (which were at the detection limit). The dilutions contained a known concentration of culture (0.5 genome copies/µL and 0.25 genome copies/µL). The four spiked samples were extracted with MagDEA Dx SV kit (Batch n°18M020, expiry date 2022-05), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time

PCR Detection Kit (Batch n° CFR1XL-EXP.469C, expiry date 2022-07) in quintupled on Bio-Rad CFX96™ Real-Time PCR Detection System. The arithmetic mean ($\bar{x}$), standard deviation (σ) and coefficient of variation (CV%) were calculated and are detailed in Table 7.

	Genome copies/ rxn (VTM, Vircell)	
Negative samples spiked with quantified Influenza A/PR/8/34 (H1N1) virus, purified (ATCC® VR95PQ™)	**5**	**2.5**
$\bar{x}$ (Ct)	34.42	35.90
σ	0.92	0.99
CV%	2.66	2.77
n	20/20	18/20

Table 7: The LoD was determined with quantified Influenza A/PR/8/34 (H1N1) virus and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -FAM channel- (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07). **rxn** = reaction, (**Ct**) = threshold cycle, ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, (***n***) = number of samples amplified.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a detection limit of ≥5 genome copies per reaction for Influenza A with a positive rate of ≥95%. This concentration was considered the LoD for Influenza A.

The LoD of Influenza B in genome copies per reaction (genome copies/rxn) was determined by testing five times four negative clinical oropharyngeal (throat) swabs (viral transport medium, VTM- Vircell-) (total twenty times) spiked with a known concentration of frozen quantified Influenza B/Florida/4/2006 virus (Yamagata Lineage), Purified (ATCC®VR1804PQ™) (which were at the detection limit). The dilutions contained a known concentration of culture (0.2 genome copies/µL and 0.10 genome copies/µL). The four spiked samples were extracted with MagDEA Dx SV kit (Batch n°18M020, expiry date 2022-05), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch n° CFR1XL-EXP.469C, expiry date 2022-07) in quintupled on Bio-Rad CFX96™ Real-Time PCR Detection System. The arithmetic mean ($\bar{x}$), standard deviation (σ) and coefficient of variation (CV%) were calculated and are detailed in Table 8.

	Genome copies/ rxn (VTM, Vircell)	
Negative samples spiked with quantified Influenza B/Florida/4/2006 virus (Yamagata Lineage), Purified (ATCC®VR1804PQ™)	**20**	**10**
$\bar{x}$ (Ct)	32.90	33.99
σ	1.81	2.24
CV%	5.51	6.58
n	19/20	10/20

Table 8: The LoD was determined with quantified Influenza B/Florida/4/2006 virus and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -FAM channel- (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07). **rxn** = reaction, (**Ct**) = threshold cycle, ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, (***n***) = number of samples amplified.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a detection limit of ≥20 genome copies per reaction for Influenza B with a positive rate of ≥95%. This concentration was considered the LoD for Influenza B.

The LoD of RSV in genome copies per reaction (genome copies/rxn) was determined by testing five times four negative clinical oropharyngeal (throat) swabs (viral transport medium, VTM- Vircell-) (total twenty times) spiked with a known concentration of frozen quantified Human Respiratory Syncytial Virus strain Long (ATCC® VR26PQ™) (which were at the detection limit). The dilutions contained a known concentration of culture (1 genome copies/µL and 0.50 genome copies/µL). The four spiked samples were extracted with MagDEA Dx SV kit (Batch nº18M020, expiry date 2022-05), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch nº CFR1XL-EXP.469C, expiry date 2022-07) in quintupled on Bio-Rad CFX96™ Real-Time PCR Detection System. The arithmetic mean ($\bar{x}$), standard deviation (σ) and coefficient of variation (CV%) were calculated and are detailed in Table 9.

	Genome copies/ rxn (VTM, Vircell)	
Negative samples spiked with quantified Human Respiratory Syncytial Virus strain Long (ATCC® VR26PQ™)	**10**	**5**
$\bar{x}$ (Ct)	34.55	34.79
σ	2.01	1.16
CV%	5.80%	3.34%
n	19/20	16/20

Table 9: The LoD was determined with quantified Human Respiratory Syncytial Virus strain Long and VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit -FAM channel- (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07). **rxn** =

reaction, (**Ct**) = threshold cycle, ($\overline{\mathbf{X}}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, (***n***) = number of samples amplified.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit showed a detection limit of ≥10 genome copies per reaction for RSV with a positive rate of ≥95%. This concentration was considered the LoD for RSV.

The results have been recorded on Excel data sheet "1 Curves-Analytical sensitivity CFR1".

3 NO TEMPLATE CONTROL ASSAY

To determine the "limit of blank" (LoB), non-template control assay was performed. For this assay, three 96 reaction mixes were reconstituted with rehydration buffer. Afterwards the Water RNAse/DNAse free was added and reactions were run on Cobas z480 Analyzer (Roche Molecular Diagnostics), AriaMx Realtime PCR System (Agilent Technologies) and DTprime Real-time Detection Thermal Cycler (DNA-Technology) (VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07).

The absence of signal in the FAM, ROX and Cy5 channels were checked; as well as the absence of signal for the Internal Control in HEX channel (due to the assay uses a human housekeeping gene as **an endogenous Internal Control (IC)**, human *RNase P gene*).

The LoB is the highest value we expect to see in a series of results on a sample that contains no analyte. Almost no signal was detected above the threshold values established in no channel. Some positive signals in HEX channel were randomly detected and accepted with a Ct value greater than 35.

The results have been recorded on Excel data sheet "1 Curves-Analytical sensitivity CFR1 (Negative sheet)".

4 PRECISION

To determine precision, intra-assay (repeatability), inter-assay (reproducibility) and inter-batch assays were performed with oropharyngeal (Throat) swabs collected in viral transport media (VTM- Vircell-). For all the assays, one positive sample for SARS-CoV-2, one positive sample for Influenza virus, one positive sample for RSV, and two additional positive and negative samples for all viruses (SARS-CoV-2, Influenza and RSV) were tested (Positive samples with different Ct values above LOD, samples with a Ct value ≤

35). SARS-CoV-2 positive specimen consisted of a pool of negative samples spiked with a known concentration of synthetic SARS-CoV-2 RNA controls (two variants MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020) and MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1) (Twist Bioscience Corporation).

The panel of samples were extracted with "Maxwell® 16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Promega) (Batch 167990) and MagDEA Dx SV kit (Batch n°18M010, expiry date 2022-04), using the magLEAD® 12gC instrument (Precision System Science Co.) following the manufacturer's instructions and were analyzed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07).

Clinical specimens were stored frozen at -20 or -80°C and were totally thawed, brought to room temperature and homogenized before testing. RNA and DNA samples were stored at -20 or -80°C until used for molecular analyses.

The arithmetic mean ($\bar{x}$), the standard deviation (σ) and the coefficient of variation (CV%) were calculated and the results are shown in Tables 10, 11 and 12.

4.1 INTRA-ASSAY

To carry out the intra-assay analysis, eight replicates of all samples were tested in the same run using VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit and DTprime Real-time Detection Thermal Cycler. In addition, in the same run, the Positive and Negative Controls (PC and NC, respectively) were also analyzed 8 times, as well as the Internal Control (IC). Ct values were obtained from the Negative sample and Negative Control. The results obtained are described below in Table 10.

4.2 INTER-ASSAY

The inter-assay values were determined by testing the different samples with different concentrations on three different days by three different operators with the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit run on the DTprime Real-time Detection Thermal Cycler. In a similar way, the Positive and Negative controls (PC and NC, respectively) were also analyzed (Table 11).

4.3 INTER-BATCH (INTERMEDIATE PRECISION)

The inter-batch values were determined with three replicates at three different sample concentrations per batch (CFR1XL-EXP.469C, CFR1XL-EXP.469D, and CFR1XL-EXP.469E) of VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit and run on the DTprime Real-time Detection Thermal Cycler. The results are described below in Table 12. In addition, the Positive and Negative Controls (PC and NC, respectively) were also analyzed in a similar way.

Sample	Pathogen	Viasure channel	$\bar{x}$ (Ct)	σ	CV %
Positive 1	SARS-CoV-2	FAM	29.42	0.36	1.21
Positive 2	Influenza Virus	ROX	29.74	0.40	1.36
Positive 3	RSV Virus	Cy5	31.93	0.20	0.61
Three viruses Positive	SARS-CoV-2	FAM	30.51	0.22	0.72
	Influenza Virus	ROX	31.64	0.28	0.89
	RSV Virus	Cy5	33.88	0.81	2.39
Negative sample	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	25.05	0.13	0.53
Positive Control	SARS-CoV-2	FAM	22.84	0.20	0.89
	Influenza Virus	ROX	26.24	0.13	0.48
	RSV Virus	Cy5	25.22	0.17	0.66
Negative Control	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 10: Intra-assay reproducibility of VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07). (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**)= standard deviation, (**CV %**)= coefficient of variation, Neg= negative, n.a.= not applicable.

Sample	Pathogen	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive 1	SARS-CoV-2	FAM	29.64	0.91	3.06
Positive 2	Influenza Virus	ROX	30.08	0.84	2.79
Positive 3	RSV Virus	Cy5	32.10	0.54	1.69
Three viruses Positive	SARS-CoV-2	FAM	30.72	0.61	1.99
	Influenza Virus	ROX	31.84	0.81	2.53
	RSV Virus	Cy5	34.43	0.90	2.63
Negative sample	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	25.03	0.19	0.76
Positive Control	SARS-CoV-2	FAM	22.78	0.17	0.77
	Influenza Virus	ROX	26.15	0.20	0.76
	RSV Virus	Cy5	25.16	0.22	0.87
Negative Control	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 11: Inter-assay reproducibility of VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07). (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**)= standard deviation, (**CV %**)= coefficient of variation, Neg= negative, n.a.= not applicable

Sample	Pathogen	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive 1	SARS-CoV-2	FAM	29.93	0.52	1.74
Positive 2	Influenza Virus	ROX	29.61	0.77	2.61
Positive 3	RSV Virus	Cy5	30.98	0.47	1.51
Three viruses Positive	SARS-CoV-2	FAM	30.20	0.24	0.81
	Influenza Virus	ROX	30.61	0.27	0.89
	RSV Virus	Cy5	31.98	0.54	1.70
Negative sample	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	23.70	0.14	0.60
Positive Control	SARS-CoV-2	FAM	22.00	0.11	0.48
	Influenza Virus	ROX	25.13	0.12	0.49
	RSV Virus	Cy5	23.40	0.12	0.49
Negative Control	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 12: Inter-bath reproducibility of VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batches n°: CFR1XL-EXP.469C, CFR1XL-EXP.469D, and CFR1XL-EXP.469E,expiry date 2022-07 (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ)= standard deviation, (**CV %**)= coefficient of variation, Neg= negative, n.a.= not applicable.

The results have been internally recorded on Excel data sheet "2 Precision CFR".

5 ANALYTICAL SPECIFICITY AND REACTIVITY

The analytical specificity and reactivity of VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit was assessed by using **publicly available nucleotide sequence database** as NCBI Genbank (https://www.ncbi.nlm.nih. gov/genbank/), in particular GenBank/SRA (https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/), and **search and/or alignment tools** as BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), Betacoronavirus BLAST (specialized BLAST page that searches Betacoronavirus sequences), and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast).

The preliminary bioinformatic analyses showed that the most of full length and good quality SARS-CoV-2 sequences published sequences in databases, were detected by at least one primers/probes set. However, mutations that might occur in these highly conserved regions (although rare) may cause RNA to be undetectable. Besides, the *in silico* analysis showed that the rest of targeted pathogens (viral and relevant RSV A and B genotypes, distinct Influenza A viral subtypes and relevant strains, as well as, both

lineages Victoria and Yamagata and relevant Influenza B virus strains) were detected by the chosen primers and probes.

The specific primer and probe combinations for detection of the N2 and N1 targets used for the detection of SARS-CoV-2, Influenza A/B and RSV A/B, do not show significant combined homologies with the human genome and human microflora, or microbial species (in particular, Human coronaviruses (HCoVs) strains: 229E, HKU1, NL63, OC43, SARS-CoV and MERS-CoV) that would predict potential false positive RT-qPCR results.

In addition, the analytical specificity for this assay was confirmed by testing a panel of different microorganisms which represents the most common respiratory pathogens. No cross-reactivity was detected between almost any of the following microorganisms tested, except the targeted pathogens of each assay (Table 13).

Cross-reactivity testing					
Human Adenovirus types 1-5, 8, 15, 31, 40 and 41	-	Influenza A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a)	-/+	Influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2) virus	-/+
Bocavirus	-	Influenza A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a)	-/+	Influenza A/Chicken/Myanmar/433/2016 (H9N2) virus	-/+
Bordetella bronchiseptica	-	Influenza A/Newcastle/607/2019 (H3N2) virus	-/+	Influenza A/Hong Kong/1073/99 (H9N2) virus	-/+
Bordetella holmesii	-	Influenza A/New York/39/2012 (H3N2) virus	-/+	Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26 virus	-/+
Bordetella parapertussis	-	Influenza A/Ohio/2/2012 (H3N2) virus	-/+	Influenza B/Brisbane/60/2008 virus	-/+
Bordetella pertussis	-	Influenza A/Perth/1001/2018 (H3N2) virus	-/+	Influenza B/Colorado/6/2017 virus	-/+
Chlamydia caviae	-	Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2) virus	-/+	Influenza B/Malaysia/2506/2004 virus	-/+
Chlamydia psittaci genotype A and C	-	Influenza A/South Australia/55/2014 (H3N2) virus	-/+	Influenza B/Maryland/15/2016 virus	-/+
Chlamydophila pneumoniae CM-1	-	Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus	-/+	Influenza B/Netherlands/207/06 virus	-/+
Human coronavirus 229E, OC43, NL63 and HKU1	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-/+	Influenza B/Netherlands/2518/2016 (clade 1A) virus	-/+
MERS Coronavirus	-	Influenza A/Texas/50/2012 (H3N2) virus	-/+	Influenza B/Nevada/3/2011 virus	-/+
SARS Coronavirus Strain Frankfurt 1	-	Influenza A/Thüringen/5/2017 (H3N2) virus (Clade 3C2a.1)	-/+	Influenza B/New Jersey/1/2012 virus	-/+
SARS-CoV-2 strain BetaCoV/Germany/BavPat1/2020 p.1	-/+	Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C) virus	-/+	Influenza B/Texas/02/2013 virus	-/+
SARS-CoV-2 strain 2019-nCoV/Italy-INMI1	-/+	Influenza A/Victoria/210/2009(H3N2) virus	-/+	Influenza B/Canberra/11/2016 virus	-/+

Cross-reactivity testing					
SARS-CoV-2 isolate Australia/VIC01/2020	-/+	Influenza A/Victoria/361/2011 (H3N2) virus	-/+	Influenza B/Florida/4/06 virus	-/+
SARS-CoV-2 isolate Wuhan-Hu-1	-/+	Influenza A/Victoria/361/2011 IVR-165 (H3N2) virus	-/+	Influenza B/Florida/07/2004 virus	-/+
SARS-CoV-2 strain 2019nCoV/USAWA1/2020	-/+	Influenza A/Anhui/01/2005 (H5N1) virus	-/+	Influenza B/Guangdong/120/2000 virus	-/+
Enterovirus 68 and 71	-	Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1) virus	-/+	Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus	-/+
Enterovirus Echovirus 11 and 30	-	Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1) virus	-/+	Influenza B/ Jiangsu/10/2003 virus	-/+
Enterovirus Coxsackievirus A24, A9 and B3	-	Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1) virus	-/+	Influenza B/Massachusetts/2/2012 virus	-/+
Haemophilus influenzae MinnA	-	Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus	-/+	Influenza B/Netherlands/365/2016 (clade 3) virus	-/+
Influenza A/PR/8/34 (H1N1) virus	-/+	Influenza A/chicken/Yunnan/1251/2003 (H5N1) virus	-/+	Influenza B/Phuket/3073/2013 virus	-/+
Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09 virus	-/+	Influenza A/common magpie/Hong Kong/645/2006 (H5N1) virus	-/+	Influenza B/Texas/06/2011 virus	-/+
Influenza A/California/7/2009(H1N1)pdm09 virus	-/+	Influenza A/duck/Hunan/795/2002 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 virus	-/+
Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/321/2007 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 BX-41A virus	-/+
Influenza A/Massachusetts/15/2013 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1) virus	-/+	*Legionella bozemanii*	-
Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1) virus	-/+	*Legionella dumoffii*	-
Influenza A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1)	-/+	Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 virus	-/+	*Legionella longbeachae*	-
Influenza A/New Caledonia/20/99(H1N1) virus	-/+	Influenza A/Hong Kong/213/2003 (H5N1) virus	-/+	*Legionella micdadei*	-
Influenza A/New York/18/2009 (H1N1)pdm09 virus	-/+	Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus	-/+	*Legionella pneumophila*	-
Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-/+	Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1) virus	-/+	Human metapneumovirus A and B	-
Influenza A/Sydney/134/2018 (H1N1)pdm09 virus	-/+	Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1) virus	-/+	*Moraxella catarrhalis*	-
Influenza A/Victoria/2040/2018 (H1N1)pdm09 virus	-/+	Influenza A/Vietnam/1194/2004 (H5N1) virus	-/+	*Mycoplasma pneumoniae*	-

Cross-reactivity testing					
Influenza A/Brisbane/117/2018 (H3N2) virus	-/+	Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1) virus	-/+	*Mycobacterium tuberculosis* not rifampin resistant	-
Influenza A/Brisbane/1028/2017 (H3N2) virus	-/+	Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1) virus	-/+	Human parainfluenza 1, 2, 3 and 4 viruses	-
Influenza A/Fujian/411/2002 (H3N2) virus	-/+	Influenza A/Whooper Swan/R65/2006 (H5N1) virus	-/+	*Pneumocystis jirovecii* Type A1 and g885652	-
Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2) virus	-/+	Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus	-/+	Human rhinovirus type C	-
Influenza A/Hong Kong/4801/2014 (H3N2) virus	-/+	Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3) virus	-/+	*Staphylococcus aureus* subsp. *aureus*	-
Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-/+	Influenza A/Duck/Lao/XBY004/2014 (H5N6) (Clade 2.3.4.4) virus	-/+	*Staphylococcus epidermidis*	-
Influenza A/Indiana/8/2011 (H3N2)v virus	-/+	Influenza A/DE-SH/Reiherente/AR8444/ 2013 (H5N8) virus	-/+	*Streptococcus pneumoniae* Z022	-
Influenza A/Indiana/10/2011 (H3N2)v virus	-/+	Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2) virus	-/+	*Streptococcus pyogenes*	-
Influenza A/Kansas/14/2017 (H3N2) virus	-/+	Influenza A/Mallard/Netherlands/2/2009 (H7N7) virus	-/+	*Streptococcus salivarius*	-
Influenza A/Kansas/14/2017, NYMC X-327 (H3N2) virus	-/+	Influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1 virus	-/+	Respiratory syncytial virus (RSV) A	-/+
Influenza A/Kumamoto/102/2002 (H3N2) virus	-/+	Influenza A/Anhui/1/2013 (H7N9) virus	-/+	Respiratory syncytial virus (RSV) B (strain CH93(18)-18)	-/+
Influenza A/Minnesota/11/2010 (H3N2)v virus	-/+	Influenza A/Guangdong/17SF00k3/2016 (H7N9) virus	-/+	Respiratory Syncytial Virus strain Long	-/+
Influenza A/Minnesota/11/2010 X203 (H3N2)v virus	-/+	Influenza B/Townsville/8/2016 virus	-/+		

Table 13: Pathogenic microorganisms of reference used in this study.

The analytical reactivity of the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit for **SARS-CoV-2** was evaluated against the following strains (as templates): RNA from Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1, Human 2019-nCoV strain 2019-nCoV/Italy-INMI1, synthetic RNA controls for two variants of the SARS-CoV-2 virus: MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020) and MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1), and heat inactivated SARS-CoV-2 strain 2019nCoV/USAWA1/2020 (ATCC® VR1986HK™).

The reactivity of the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit for **Influenza A** was evaluated against the following strains (as templates):

Influenza A/PR/8/34 (**H1N1**) virus

Influenza A/Brisbane/02/2018, IVR-190 (**H1N1**)pdm09 virus

Influenza A/California/7/2009(**H1N1**)pdm09 virus

Influenza A/Dominican Republic/7293/2013 (**H1N1**)pdm09 virus

Influenza A/Massachusetts/15/2013 (**H1N1**)pdm09 virus

Influenza A/Michigan/45/2015 (**H1N1**)pdm09 virus

Influenza A/Netherlands/1250/2016 (**H1N1**)pdm09 virus (clade 6B.1)

Influenza A/New Caledonia/20/99(**H1N1**) virus

Influenza A/New York/18/2009 (**H1N1**)pdm09 virus

Influenza A/Singapore/GP1908/2015 virus, IVR-180 (**H1N1**)pdm09 virus

Influenza A/Sydney/134/2018 (**H1N1**)pdm09 virus

Influenza A/Victoria/2040/2018 (**H1N1**)pdm09 virus

Influenza A/Brisbane/117/2018 (**H3N2**) virus

Influenza A/Brisbane/1028/2017 (**H3N2**) virus

Influenza A/Fujian/411/2002 (**H3N2**) virus

Influenza A/Hiroshima//52/2005 (IVR-142) (**H3N2**) virus

Influenza A/Hong Kong/4801/2014 (**H3N2**) virus

Influenza A/Hong Kong/4801/2014 NYMC X-263B (**H3N2**) virus

Influenza A/Indiana/8/2011 (**H3N2**)v virus

Influenza A/Indiana/10/2011 (**H3N2**)v virus

Influenza A/Kansas/14/2017 (**H3N2**) virus

Influenza A/Kansas/14/2017, NYMC X-327 (**H3N2**) virus

Influenza A/Kumamoto/102/2002 (**H3N2**) virus

Influenza A/Minnesota/11/2010 (**H3N2**)v virus

Influenza A/Minnesota/11/2010 X203 (**H3N2**)v virus

Influenza A/Netherlands/398/2014 (**H3N2**) virus (clade 3C.3a)

Influenza A/Netherlands/2393/2015 (**H3N2**) virus (clade 3C.2a)

Influenza A/Newcastle/607/2019 (**H3N2**) virus

Influenza A/New York/39/2012 (**H3N2**) virus

Influenza A/Ohio/2/2012 (**H3N2**) virus

Influenza A/Perth/1001/2018 (**H3N2**) virus

Influenza A/Singapore/INFIMH-16-0019/2016 (**H3N2**) virus

Influenza A/South Australia/55/2014 (**H3N2**) virus

Influenza A/South Australia/55/2014, IVR-175 (**H3N2**) virus

Influenza A/Switzerland/9715293/2013 (**H3N2**) virus

Influenza A/Texas/50/2012 (**H3N2**) virus

Influenza A/Thüringen/5/2017 (**H3N2**) virus (Clade 3C2a.1)

Influenza A/Uruguay/716/2007 (**H3N2**)(NYMC X-175C) virus

Influenza A/Victoria/210/2009(**H3N2**) virus

Influenza A/Victoria/361/2011 (**H3N2**) virus

Influenza A/Victoria/361/2011 IVR-165 (**H3N2**) virus

Influenza A/Anhui/01/2005 (**H5N1**) virus

Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (**H5N1**) virus

Influenza A/chicken/Vietnam/NCVD-016/2008 (**H5N1**) virus

Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (**H5N1**) virus

Influenza A/chicken/Vietnam/NCVD-03/08 (**H5N1**) - PR8-IDCDC-RG25a virus

Influenza A/chicken/Yunnan/1251/2003 (**H5N1**) virus

Influenza A/common magpie/Hong Kong/645/2006 (**H5N1**) virus

Influenza A/duck/Hunan/795/2002 (**H5N1**) virus

Influenza A/Egypt/321/2007 (**H5N1**) virus

Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (**H5N1**) virus

Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (**H5N1**) virus

Influenza A/Egypt/N03072/2010 (**H5N1**) x PR8-IDCDC-RG29 virus

Influenza A/Hong Kong/213/2003 (**H5N1**) virus

Influenza A/Hubei/1/2010 (**H5N1**) x PR8-IDCDCRG30 virus

Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (**H5N1**) virus

Influenza A/Japanese white eye/Hong Kong/1038/2006 (**H5N1**) virus

Influenza A/Vietnam/1194/2004 (**H5N1**) virus

Influenza A/Vietnam/1194/2004 (NIBRG-14) (**H5N1**) virus

Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (**H5N1**) virus

Influenza A/Whooper Swan/R65/2006 (**H5N1**) virus

Influenza A/pheasant/New Jersey/1355/1998 (**H5N2**)-PR8-IBCDC-4 virus

Influenza A/Duck/Singapore-Q/F119-3/97 (**H5N3**) virus

Influenza A/Duck/Lao/XBY004/2014 (**H5N6**) virus (Clade 2.3.4.4)

Influenza A/DE-SH/Reiherente/AR8444/ 2016 (**H5N8**) virus

Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (**H7N2**) virus

Influenza A/Mallard/Netherlands/2/2009 (**H7N7**) virus

Influenza A/Mallard/Netherlands/12/2000 (**H7N7**) - IBCDC-1 virus

Influenza A/Anhui/1/2013 (**H7N9**) virus

Influenza A/Guangdong/17SF003/2016 (**H7N9**) virus

Influenza A/Chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (**H9N2**) virus

Influenza A/Chicken/Myanmar/433/2016 (**H9N2**) virus

Influenza A/Hong Kong/1073/99 (**H9N2**) virus

Influenza A/Hong Kong/33982/2009 (**H9N2**) x PR8-IDCDC-RG26 virus

The reactivity of the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit for **Influenza B** was evaluated against the following strains (as templates):

Influenza B/Brisbane/60/2008 virus (**B/Victoria lineage**)

Influenza B/Colorado/6/2017 virus (**B/Victoria lineage**)

Influenza B/Malaysia/2506/2004 virus (**B/Victoria lineage**)

Influenza B/Maryland/15/2016 virus (**B/Victoria lineage**)

Influenza B/Netherlands/207/06 virus (**B/Victoria lineage**)

Influenza B/Netherlands/2518/2016 (clade 1A) virus (**B/Victoria lineage**)

Influenza B/Nevada/3/2011 virus (**B/Victoria lineage**)

Influenza B/New Jersey/1/2012 virus (**B/Victoria lineage**)

Influenza B/Texas/02/2013 virus (**B/Victoria lineage**)

Influenza B/Townsville/8/2016 virus (**B/Victoria lineage**)

Influenza B/Canberra/11/2016 virus (**B/Yamagata lineage**)

Influenza B/Florida/4/06 virus (**B/Yamagata lineage**)

Influenza B/Florida/07/2004 virus (**B/Yamagata lineage**)

Influenza B/Guangdong/120/2000 virus (**B/Yamagata lineage**)

Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus (**B/Yamagata lineage**)

Influenza B/Jiangsu/10/2003 virus (**B/Yamagata lineage**)

Influenza B/Massachusetts/2/2012 virus (**B/Yamagata lineage**)

Influenza B/Netherlands/365/2016 (clade 3) virus (**B/Yamagata lineage**)

Influenza B/Phuket/3073/2013 virus (**B/Yamagata lineage**)

Influenza B/Texas/06/2011 virus (**B/Yamagata lineage**)

Influenza B/Wisconsin/1/2010 virus (**B/Yamagata lineage**)

Influenza B/Wisconsin/1/2010 BX-41A virus (**B/Yamagata lineage**)

The reactivity of the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit for **RSV** was confirmed against RSV strain Long, RSV type A and RSV type B (strain CH93(18)-18), (as template).

Analytical specificity and reactivity of the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit were evaluated with Batch nº: CFR1XL-EXP.469C, CFR1XL-EXP.469D and CFR1XL-EXP.469E (Expiry dates 2022-07, 2022-07 and 2022-07). These assays were run on Bio-Rad CFX96™ Real-Time PCR Detection System.

The results have been recorded on Excel data sheet "3 Cross-reactivity testing - Reactivity and Specificity CFR1".

6 METROLOGICAL TRACEABILITY

The metrological traceability of VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit has been evaluated with different types of controls:

1. Synthetic cDNA fragments: reference material that we use to evaluate the different batches of the product. The synthetic cDNA fragments have been designed for each reaction and each pathogen:
    - Name of synthetic cDNA fragment for N1 and N2 targets SARS-CoV-2: NCO7PC and NCO9PC, FAM channel.
    - Name of synthetic cDNA fragment for Influenza A, A (H1N1)pdm09 and B: YIAXPC, HNVXPC, YIBXPC (respectively), ROX channel.

- Name of synthetic cDNA fragment for RSV A and B: RSAXPC and RSBXPC (respectively), Cy5 channel.

For the design of the synthetic fragments, it has been considered the amplicon, primers, and probes.

2. Reference strains: the validity of obtained results during the product validation has been verified using different reference strains for each pathogen. Strains used:
   - Human Respiratory Syncytial Virus strain Long (ATCC, Ref: ATCC® VR26PQ™).
   - SARS-CoV-2: Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1 (EVAg, Ref. 026N-03889).
   - Human 2019-nCoV strain 2019-nCoV/Italy-INMI1 (EVAg, Ref: 008N-03894)
   - Synthetic RNA controls for two variants of the SARS-CoV-2 virus: MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020) and MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1) (Twist Bioscience Corporation, Ref 102019 and 102024: Twist Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1) and 2 (MN908947.3)).
   - Heat inactivated SARS-CoV-2 strain 2019nCoV/USAWA1/2020 (ATCC® VR1986HK™).
   - National Institute for Biological Standards and Control (NIBSC). NIBSC plays a major role in assuring the quality of biological medicines worldwide through the provision of biological reference materials, by testing products and carrying out research. NIBSC have a leading international role in preparing, evaluating and distributing international biological standards and other biological reference materials.
   - The International Reagent Resource (IRR). IRR is a biological reagent repository established in 2008 by the Centers for Disease Control and Prevention (CDC) Influenza Division which contracted with The American Type Culture Collection ("ATCC®"). IRR provides registered users with reagents, tools, and information for studying and detection of Influenza Virus. In this regard, the IRR produces reagents for researching and developing improved diagnostic tests and, hence, to prepare for a future pandemic. For this reason, the material produced is in connection with an international framework called the "PIP Framework", Pandemic Influenza Preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits, approved by all the Member countries of the World Health Organization (WHO) in May 2011.
   - The American Type Culture Collection ("ATCC®"). The American Type Culture Collection (ATCC) is a private, nonprofit organization dedicated to the acquisition, preservation, authentication, and distribution of diverse biological materials. ATCC was founded by scientists in 1925 to serve as a national repository and distribution center for cultures of microorganisms. Since that time, viruses, animal and plant cell cultures, and recombinant DNA materials have been added. ATCC is now the largest general service culture

collection in the world, with collections in six areas: Bacteriology, Cell Culture, Molecular Biology, Mycology, Protistology, and Virology.

External Reference	Pathogen Name	Variety	Procedence
ATCC® VR95PQ™	Influenza (H1N1) virus	Influenza A/PR/8/34 (H1N1) virus	ATCC
15/252	Influenza A (H1N1 pdm09)	A/California/7/2009 (H1N1 pdm09)	NIBSC
17/264	Influenza A (H1N1 pdm09)	A/Michigan/45/2015 (H1N1 pdm09)	NIBSC
16/292	Influenza A (H1N1 pdm09)	A/Singapore/GP1908/2015 virus, IVR-180 (H1N1)pdm09	NIBSC
FR-1300	Influenza A (H1N1 pdm09)	A/Dominican Republic/7293/2013 (H1N1 pdm09) V.Retained at ATCC	ATCC (IRR)
FR-1325	Influenza A (H1N1 pdm09)	A/Massachussets/15/2013 (H1N1 pdm09) V.Retained at ATCC	ATCC (IRR)
18/236	Influenza A (H1N1 pdm09)	A/Brisbane/02/2018 (H1N1)pdm09	NIBSC
18/242	Influenza A (H3N2)	A/Kansas/14/2017 (H3N2)	NIBSC
14/224	Influenza A (H3N2)	A/Switzerland/9715293/2013 (H3N2)	NIBSC
15/192	Influenza A (H3N2)	A/Hong Kong/4801/2014 (H3N2)	NIBSC
17/196	Influenza A (H3N2)	A/Singapore/INFIMH-16-0019/2016 (H3N2)	NIBSC
14/226	Influenza A (H3N2)	A/South Australia/55/2014 (H3N2)	NIBSC
08/278	Influenza A (H3N2)	A/Uruguay/716/2007 (H3N2)	NIBSC
FR-983	Influenza A (H3N2)	A/Minenesota/11/2010 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-984	Influenza A (H3N2)	A/Minenesota/11/2010 X203 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-982	Influenza A (H3N2)	A/Indiana/8/2011 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-985	Influenza A (H3N2)	A/Indiana/10/2011 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-986	Influenza A (H3N2)	A/Indiana/10/2011 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-1042	Influenza A (H3N2)	A/Victoria/361/2011 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-1095	Influenza A (H3N2)	A/Victoria/361/2011 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-1057	Influenza A (H3N2)	A/Victoria/361/2011 IVR-165 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-1148	Influenza A (H3N2)	A/Fujian/411/2002 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-1145	Influenza A (H3N2)	A/Ohio/2/2012 (H3N2) V.Retained at ATCC	ATCC (IRR)
FR-1212	Influenza A (H3N2)	A/Texas/50/2012 (H3N2).Retained at ATCC	ATCC (IRR)
FR-1309	Influenza A (H3N2)	A/New York/39/2012 (H3N2)	ATCC (IRR)
03/198	Influenza A (H3N2)	A/Kumamoto/102/2002 (H3N2)	NIBSC
05/234	Influenza A (H3N2)	A/Hiroshima//52/2005 (IVR-142) (H3N2)	NIBSC
FR-273	Influenza A (H5N1)	A/chicken/Vietnam/NCVD-016/2008 (H5N1)	ATCC (IRR)
FR-878	Influenza A (H5N1)	A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1)	ATCC (IRR)

External Reference	Pathogen Name	Variety	Procedence
FR-879	Influenza A (H5N1)	A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a	ATCC (IRR)
FR-880	Influenza A (H5N1)	A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1)	ATCC (IRR)
FR-881	Influenza A (H5N1)	A/Egypt/3300-NAMRU3/2008 x PR8- IDCDC-RG13 (H5N1)	ATCC (IRR)
FR-1071	Influenza A (H5N1)	A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29	ATCC (IRR)
FR-266	Influenza A (H5N1)	A/Egypt/321/2007 (H5N1)	ATCC (IRR)
FR-874	Influenza A (H5N1)	A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1)	ATCC (IRR)
09/184	Influenza A (H5N1)	A/Vietnam/1194/2004 (H5N1) (NIBRG-14)	NIBSC
FR-264	Influenza A (H5N1)	A/Vietnam/1194/2004 (H5N1)	ATCC (IRR)
FR-893	Influenza A (H5N1)	A/India/NIV/2006 x PR8-IBCDC-RG7 (H5N1)	ATCC (IRR)
FR-263	Influenza A (H5N1)	A/chicken/Yunnan/1251/2003 (H5N1)	ATCC (IRR)
FR-265	Influenza A (H5N1)	A/duck/Hunan/795/2002	ATCC (IRR)
FR-271	Influenza A (H5N1)	A/Anhui/01/2005 (H5N1)	ATCC (IRR)
FR-873	Influenza A (H5N1)	A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1)	ATCC (IRR)
FR-270	Influenza A (H5N1)	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	ATCC (IRR)
FR-269	Influenza A (H5N1)	A/common magpie/Hong Kong/645/2006 (H5N1)	ATCC (IRR)
FR-1072	Influenza A (H5N1)	A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30	ATCC (IRR)
FR-883	Influenza A (H5N2)	A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4. Retained at ATCC	ATCC (IRR)
00/552	Influenza A (H5N3)	A/Duck/Singapore-Q/F119-3/97 (H5N3)	NIBSC
FR-894	Influenza A (H7N2)	A/Turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2). Retained at ATCC	ATCC (IRR)
FR-884	Influenza A (H7N7)	A/Mallard/Netherlands/12/2000 (H7N7)-IBCDC-1. Retained at ATCC	ATCC (IRR)
FR-1249	Influenza A (H7N9)	A/Anhui/1/2013 (H7N9).Retained at ATCC	ATCC (IRR)
FR-885	Influenza A (H9N2)	A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2). Retained at ATCC Exp.01/28/2019	ATCC (IRR)
FR-886	Influenza A (H9N2)	A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26	ATCC (IRR)
08/208	Influenza A (H9N2)	A/Hong Kong/1073/99 (H9N2)	NIBSC
15/146	Influenza B	B/Brisbane/60/2008	NIBSC
13/234	Influenza B	B/Brisbane/60/2008	NIBSC
FR-1048	Influenza B	B/Nevada/3/2011 (BV).Retained at ATCC	ATCC (IRR)
FR-1327	Influenza B	B/Texas/02/2013 (BV).Retained at ATCC	ATCC (IRR)
FR-1304	Influenza B	B/Texas/02/2013 (BV).Retained at ATCC	ATCC (IRR)
FR-1272	Influenza B	B/New Jersey/01/2012 (BV).Retained at ATCC	ATCC (IRR)
17/254	Influenza B	B/Colorado/06/2017	NIBSC
18/100	Influenza B	B/Maryland/15/2016	NIBSC
08/184	Influenza B	B/Malaysia/2506/2004	NIBSC
07/132	Influenza B	B/Malaysia/2506/2004	NIBSC

External Reference	Pathogen Name	Variety	Procedence
ATCC® VR1804PQ™	Influenza B	B/Florida/4/2006	ATCC
FR-1178	Influenza B	B/Florida/07/2004 (BY).Retained at ATCC	ATCC (IRR)
FR-1198	Influenza B	B/Massachusetts/02/2012 (BY).Retained at ATCC	ATCC (IRR)
FR-1096	Influenza B	B/Texas/06/2011 (BY).Retained at ATCC	ATCC (IRR)
FR-1044	Influenza B	B/Wisconsin/1/2010 (BY). Retained at ATCC	ATCC (IRR)
FR-1051	Influenza B	B/Wisconsin/1/2010 BX-41A (BY).Retained at ATCC	ATCC (IRR)
01/546	Influenza B	B/Guangdong/120/2000	NIBSC
04/202	Influenza B	B/Jiangsu/10/2003	NIBSC
12/106	Influenza B	B/Hubei Wujiagang/158/2009 (NYMC BX-39)	NIBSC

Table 14: Influenza reference strains.

3. Samples from EQA programmes: in addition to the methods cited above, the metrological traceability will be evaluated through participating in External Quality Assurance (EQA) programmes of different international independent organizations. Several laboratories participate in these programmes and results are compared among them.
    - **QCMD**: Quality Control for Molecular Diagnostics is accredited to the EN ISO/IEC 17043:2010 Standards (Conformity assessment - General requirements for proficiency testing) and by UKAS (UK's National Accreditation). All panel components from QCMD were produced and certified according to the guidelines for the production and certification of BCR reference materials (EU document BCR/48/93).
    - **INSTAND e.V.**: *Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V.* is accredited in accordance with DIN EN ISO/IEC 17043:2010.
    - **UK NEQAS for Microbiology:** UK NEQAS External Quality Assessment Services is accredited to the EN ISO/IEC 17043:2010 Standards (Conformity assessment - General requirements for proficiency testing) and by UKAS (UK's National Accreditation).
    - **RCPAQAP:** The Royal College of Pathologists of Australasia - Quality Assurance Programs overall accreditation is ISO 9001:2015-Quality Management Systems. Each of the programs is individually accredited by The National Association of Testing Authorities (NATA) and complies with the requirements of ISO/IEC 17043:2010.
    - **CAP (College of American Pathologists)** is accredited for compliance with ISO/IEC 17043.
    - **LGC, LGC Standards Proficiency Testing** is a major international provider of UKAS accredited proficiency testing services, is accredited in accordance with International Standard ISO/IEC 17043:2010, and ISO 9001:2015.
    - **LABQUALITY** is an independent, unbiased, Finnish service provider. Labquality's main quality assessment schemes are accredited in accordance with the standard ISO 17043 (FINAS, PT02, ISO 17043:2010). Labquality's management system has received ISO 9001

certification (DQS), and Labquality's certification activities are overseen by an independent and unbiased Quality Council.

All the results obtained will be described in Section 8. External Quality Assessment Programs in this report.

7 CLINICAL SENSITIVITY AND SPECIFICITY

In order to determine the clinical diagnostic accuracy of "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit", a Multicenter Evaluation will be conducted through collaboration with the National&International Microbiology Departments from different entities.

7.1 INTERNAL STUDIES

- **Cross-reactivity evaluation of the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit focused on another Human coronavirus.**

Besides, the leftovers of 17 diagnosed respiratory samples from patients with clinical suspicion of respiratory disease due viral infection (clinical sample, CS) and 7 specimens from EQA programs (EQAS), were analyzed. The clinical samples were previously analyzed with "CLART® PneumoVir2" (Genomica) which allows the detection and genotyping of multiple viruses that cause respiratory infections or with commercial qPCR test: FTD HCoV (Fast Track Diagnostics, Ref: FTD-56,1-32, Batch: COR19-32-01, 2020-05). These specimens included positives samples for Coronavirus 229E (2CS and 2 EQAS), Coronavirus NL63 (5 CS), Coronavirus OC43 (4 CS), Coronavirus HKUI (1CS and 1 EQAS), MERS Coronavirus (4 EQAS), and 5 negative specimens. The nucleic acids were extracted with MagDEA Dx SV kit (Batch nº98M020, 94M010, 92M010, 89M030), using the magLEAD® 12gC instrument (Precision System Science Co.) following manufacturer's instructions; Viasure RNA-DNA Extraction kit (Batch nº TA170039, VIASURE); Maxwell® 16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Batch nº Lot:120981, Promega); and Total Nucleic Acid Isolation (TNAI) Kit, using COBAS® AmpliPrep (ROCHE). All samples were detected correctly as negative.

- **Molecular diagnostic comparative study in collaboration with Service of Microbiology from Hospital Clínico Universitario Lozano Blesa (Zaragoza, Spain).**

In order to determine the clinical diagnostic accuracy of "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit", a retrospective comparative study was carried out in 99 respiratory specimens (oropharyngeal swabs) collected mainly in Viral Transport Medium (VTM) 2 ml (Ref: TM011(which includes 1 sterile Rayon swab), Vircell S.L., Spain), from symptomatic patients of viral respiratory infection. The

respiratory specimens were collected at the Service of Microbiology from Hospital Clínico Universitario Lozano Blesa (Zaragoza, Spain) during the winter season 2017-2018.

The leftovers of 99 diagnosed respiratory samples and negative specimens, from patients with clinical suspicion of respiratory disease due viral infection were included. These samples were previously analyzed with "CLART® PneumoVir2" (Genomica) which allows the detection and genotyping of multiple viruses that cause respiratory infections and/or with cobas® Influenza A/B & RSV with cobas® Liat® PCR System (Roche, Switzerland)) (both routine method established at the Hospital). RNA extractions were carried out using Total Nucleic Acid Isolation (TNAI) Kit, using COBAS®AmpliPrep Instrument (Roche) and/or Maxwell® 16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Promega)(Batch nº 168046). Once the RNAs were extracted, they were immediately used for molecular analyses, and after that stored at -20ºC. These samples were tested with VIASURE multiplex assay for the detection of SARS-CoV-2, Influenza A, Influenza B and RSV A and B (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07). The assays were run on AriaMx Realtime PCR System (Agilent Technologies). The results were compared with the data obtained by cobas® Influenza A/B & RSV (Roche) (Table 15).

The statistical values calculation was based on the *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests* (FDA Guide March 2007). The values were calculated by Microsoft Excel 2010 for Windows and the online resource GraphPad QuickCalcs. The 95% CI were calculated using the modified Wald method. The results are described below in Table 16.

	cobas® Influenza A/B & RSV (Roche)			
	Influenza A/B			
		+	-	Total
VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit	+	53	1*#	54
	-	4*†	41	45
	Total	57	42	99
	RSV			
		+	-	Total
	+	5	0	5
	-	0	94	94
	Total	5	94	99

Table 15. Comparative results obtained with 99 clinical samples using two different molecular diagnostic kits, VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit and cobas® Influenza A/B & RSV (Roche).

** The low amount of template RNA in this respiratory sample is below the detection limit of the method used. Besides, 1# Influenza A and 2/4t Influenzas B samples had been able to be evaluated by an additional commercial molecular detection method (FTD Respiratory pathogens 21 (Fast Track Diagnostics)), confirming our result.*

	VIASURE values vs Roche values		
Microorganism	**PPA (%)**	**NPA (%)**	**OPA (%)**
Flu A/B	93.0 (82.9 to 97.8)	97.6 (86.6 to >99.9)	95.0 (88.4 to 98.1)
RSV A/B	100 (51.1 to 100)	100 (95.3 to 100)	100 (95.5 to 100)

Table 16. The positive percentage agreement (PPA), the negative percentage agreement (NPA) and the overall percentage agreement (OPA) for VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kit (95% confidence interval).

- **Comparative performance analysis of VIASURE *SARS-CoV-2, Flu & RSV*, and other VIASURE Real Time PCR Detection kits from CerTest Biotec using SARS-CoV-2 positive clinical samples from Central BioHub.**

The clinical performance of VIASURE *SARS-CoV-2, Flu & RSV,* Real Time PCR Detection Kit was tested using 16 respiratory clinical samples (nasopharyngeal swabs collected in BD™ Universal Viral Transport System) bought through the Central BioHub Biospecimens Online company (https://www.centralbiohub.com/). All samples were bough as SARS-CoV-2 positive by real-time PCR. The sixteen samples are from symptomatic COVID-19 disease female/male donors. All of them were collected in April 2020 in New York (United States).

Clinical samples were extracted with QIAamp Viral RNA Mini (Qiagen, Lot No. 160051290) following manufacturer's instructions and analyzed with VIASURE *SARS-CoV-2, Flu & RSV* (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07), VIASURE *SARS-CoV-2 S gene* (Batch nº: NCO1XL-006, expiry date 2022-04), VIASURE *SARS-CoV-2* (Batch nº: NCO2XL-057, expiry date 2022-04) and VIASURE *SARS-CoV-2 (N1+N2)* Real Time PCR Detection kits (Batch nº: NCO3XL-EXP.459B, expiry date 2022-06) using Applied Biosystems 7500 Fast Real-Time PCR System and AriaMx Realtime PCR System (Agilent Technologies).

The statistical values calculation was based on the *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests* (FDA Guide March 2007). The values were calculated by Microsoft Excel 2010 for Windows and the online resource GraphPad QuickCalcs. The 95% CI were calculated using the modified Wald method.

12 SARS-CoV-2 positive samples were found with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit and these results were in agreement with a RT-qPCR test developed according to the China CDC Primers and probes for detection 2019-nCoV (VIASURE *SARS-CoV-2* Real Time PCR Detection kit). The results were as follows (Table 17):

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit	RT-qPCR test based on China CDC Protocol			
		+	-	Total
	+	12	0	12
	-	1*	3	4
	Total	13	3	16

Table 17. Comparative results of SARS-CoV-2.

** The low amount of template RNA in this respiratory sample is below the detection limit of the method used.*

The positive percentage agreement (PPA), the negative percentage agreement (NPA) and the overall percentage agreement (OPA) for VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit in relation to the results from China CDC assay were calculated with 95% confidence interval (CI).

The results are described below in Table 18.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit			
Microorganism	**PPA (%)**	**NPA (%)**	**OPA (%)**
SARS-CoV-2	92.3 (64.6 to >99.9)	100 (38.2 to 100)	93.8 (69.7 to >99.9)

Table 18. PPA, NPA and OPA values for VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kit (95% confidence interval).

7.2 EXTERNAL STUDIES

- **Evaluation and validation of the prototype molecular assay VIASURE SARS-CoV-2, Flu & RSV Real Time PCR Detection kit from CerTest Biotec, at Liverpool Clinical Laboratories (UK).**

The aim of this comparative-retrospective study was to evaluate and validate the prototype VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit in comparison with the reference assay SARS-CoV-2 Assay (Panther Fusion® System, Hologic) for de detection of SARS-CoV-2 and with a validated respiratory in-house RT-PCR from Liverpool Clinical Laboratories for the detection of RSV and Influenza viruses.

The leftover Flu-positive respiratory clinical samples collected during the flu season 2018-2019 and SARS-CoV-2-positive respiratory specimens collected during COVID-2019 pandemic were stored both at -20 ˚C until this comparative study had been conducted.

RNA from leftover respiratory specimens was isolated with QIAsymphony® RNA kit, using the QIAsymphony SP instrument (QIAGEN) and tested with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR

Detection kit (Batch n° CFR196TRUO-001). Analysis was performed using LightCycler® 480II thermocycler (Roche Life Science).

The endogenous internal control (IC) may or may not show an amplification signal. IC signal was obtained in 154 samples and was not observed in 13 samples. These last samples were deleted from the study since the lack of signal in any channel could indicate that the Nucleic acids had degraded and therefore were not suitable for the comparison.

Finally, a total of 154 RNAs from respiratory clinical samples were analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kits and results were compared with the reference molecular assays. Among them, 37 samples were characterized as negative for all three pathogens, 26 samples positive for SARS-CoV-2, 56 samples positives for Flu (43 Flu A including 2 samples characterized as H1N1 and 13 Flu B) and 35 positives for RSV. The overall agreement among VIASURE assay and the reference methods was 98.70% (Table 19 and 20).

The statistical values calculation was based on the *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests* (FDA Guide March 2007). The values were calculated by Microsoft Excel 2010 for Windows and the online resource GraphPad QuickCalcs. The 95% CI were calculated using the modified Wald method. The results are described below in Table 19 and 20.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit	**Reference molecular methods**			
	Influenza A/B - In-house RT-PCR			
		+	**-**	**Total**
	+	55	0	55
	-	1*	98	99
	Total	56	98	154
	RSV A/B - Reference molecular methods			
		+	**-**	**Total**
	+	34	0	34
	-	1*	119	120
	Total	35	119	154
	SARS-CoV-2 - Panther Fusion® SARS-CoV-2 Assay			
		+	**-**	**Total**
	+	26	0	26
	-	0	128	128
	Total	26	128	154

Table 19. Comparative results obtained with 154 clinical samples using two different molecular diagnostic kits, VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit and Panther Fusion® SARS-CoV-2 Assay for SARS-CoV-2 or In-house RT-PCR for Flu A/B and RSV A/B.

** The low amount of template RNA in these respiratory samples is below the detection limit of the method used. The leftover samples were storage at -20ºC and not retested by the reference assay, therefore viral RNA degradation could not be discarded.*

	VIASURE values vs Reference molecular methods		
Microorganism	**PPA (%)**	**NPA (%)**	**OPA (%)**
SARS-CoV-2	100 (84.8 to 100)	100 (96.5 to 100)	100 (97.1 to 100)
Flu A/B	98.2 (89.7 to >99.9)	100 (95.5 to 100)	99.4 (96.1 to >99.9)
RSV A/B	97.1 (84.2 to >99.9)	100 (96.2 to 100)	99.4 (96.1 to >99.9)

Table 20. PPA, NPA and OPA values for VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kit (95% confidence interval).

- **Evaluation and validation of the prototype molecular assay VIASURE SARS-CoV-2, Flu & RSV Real Time PCR Detection kit from CerTest Biotec, at Oxford University Hospitals, NHS Foundation Trust (UK).**

The aim of this comparative-retrospective study was to evaluate and validate the prototype VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit in comparison with the reference assay SARS-CoV-2 Assay (Panther Fusion® System, Hologic) for de detection of SARS-CoV-2 and with Xpert® Xpress Flu/RSV (Cepheid) for the detection of RSV and Influenza viruses.

The leftover Flu-positive respiratory clinical samples collected during the flu season 2018-2019 and SARS-CoV-2-positive respiratory specimens collected during COVID-2019 pandemic were stored both at -20 ˚C until this comparative study had been conducted.

RNA from leftover respiratory specimens was isolated with QIAsymphony® RNA kit, using the QIAsymphony SP instrument (QIAGEN) and tested with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit (Batch nº CFR196TRUO-001). Analysis was performed using Rotor-Gene Q 5plex Platform (QIAGEN). The endogenous internal control (IC) showed an amplification signal in all samples tested.

Finally, a total of 40 RNAs from respiratory clinical samples were analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kits and results were compared with the reference molecular assays. In the study it was included 10 samples positive for SARS-CoV-2, 20 samples positives for Flu (10 Flu A and 10 Flu B) and 10 positives for RSV. The samples were analyzed in duplicate. The overall agreement among VIASURE assay and the reference methods was 95%.

The statistical values calculation was based on the *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests* (FDA Guide March 2007). The values were calculated by Microsoft Excel 2010

for Windows and the online resource GraphPad QuickCalcs. The 95% CI were calculated using the modified Wald method. The results are described below in Table 21 and 22.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit	**Reference molecular methods**			
	Influenza A/B - Xpert® Xpress Flu/RSV (Cepheid)			
		+	**-**	**Total**
	+	17	0	18
	-	3*#	20	22
	Total	20	20	40
	RSV A/B - Xpert® Xpress Flu/RSV (Cepheid)			
		+	**-**	**Total**
	+	8	0	8
	-	2*¥	30	32
	Total	10	30	40
	SARS-CoV-2 - Panther Fusion® SARS-CoV-2 Assay			
		+	**-**	**Total**
	+	10	0	10
	-	0	30	30
	Total	10	30	40

Table 21. Comparative results obtained with 40 clinical samples using two different molecular diagnostic kits, VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit and Panther Fusion® SARS-CoV-2 Assay for SARS-CoV-2 or Xpert® Xpress Flu/RSV (Cepheid) for Flu A/B and RSV A/B.

** The low amount of template RNA in these respiratory samples is below the detection limit of the method used. The leftover samples were storage at -20ºC and not retested by the reference assay, therefore viral RNA degradation could not be discarded.*

#One of these 3 samples was correctly detected in one of the two assays performed in parallel. It was finally considered as True Positive.

¥ One of these 2 samples was correctly detected in one of the two assays performed in parallel. It was finally considered as True Positive.

	VIASURE values vs Reference molecular methods		
Microorganism	**PPA (%)**	**NPA (%)**	**OPA (%)**
SARS-CoV-2	100 (67.91 to 100)	100 (86.53 to 100)	100 (89.56 to 100)
Flu A/B	90.00 (68.68 to 98.43)	100 (81.02 to 100)	95.00 (82.61 to 99.5)
RSV A/B	90.00 (57.40 to >99.99)	100 (86.53 to 100)	97.50 (85.96 to >99.99)

Table 22. PPA, NPA and OPA values for VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kit (95% confidence interval).

- **Evaluation and validation of the prototype molecular assay VIASURE SARS-CoV-2, Flu & RSV Real Time PCR Detection kit from CerTest Biotec, at Royal Cornwall Hospital NHS Trust (UK).**

The aim of this comparative-retrospective study was to evaluate and validate the prototype VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit in comparison with the reference assay SARS-CoV-2 Assay (Panther Fusion® System, Hologic) and VIASURE *SARS-CoV-2* Real Time PCR detection kit (CerTest) for de detection of SARS-CoV-2; and **with** Xpert® Xpress Flu/RSV (Cepheid) and AusDiagnostics Respiratory assays for the detection of RSV and Influenza viruses.

The leftover Flu-positive respiratory clinical samples (nasal, pernasal, throat, combined nose and throat swabs and naso-pharyngeal aspirate specimens) collected during the flu season 2018-2019 and SARS-CoV-2-positive respiratory specimens collected during COVID-2019 pandemic were stored both at -20 ˚C until this comparative study had been conducted. The collection of these clinical samples was performed in 1 mL of Universal transport medium UTM® (Copan Diagnostics) or saline medium. In addition to clinical samples, specimens from QCMD SARS-CoV-2, UKNEQAS and CAP IDR programmes were added in this evaluation.

RNA from leftover respiratory specimens was isolated with the QIAamp® Viral RNA Mini Kit, using QIAcube (QIAGEN) and NucliSENS® easyMAG® (bioMérieux) and tested with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit (Batch n° CFR196TRUO-001). Analysis was performed 7500 Fast Real-Time PCR system (Applied Biosystems).

Finally, a total of 78 RNAs from respiratory clinical samples were analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kits and results were compared with the reference molecular assays. In the study it was 19 samples positive for SARS-CoV-2, 45 samples positives for Flu and 14 positives for RSV. The overall agreement among VIASURE assay and the reference methods was 96.1%.

The statistical values calculation was based on the *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests* (FDA Guide March 2007). The values were calculated by Microsoft Excel 2010 for Windows and the online resource GraphPad QuickCalcs. The 95% CI were calculated using the modified Wald method. The results are described below in Table 23 and 24.

	Reference molecular methods			
VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit	**Influenza A/B - Xpert® Xpress Flu/RSV (Cepheid) and AusDiagnostics Respiratory assays**			
		+	**-**	**Total**
	+	43	0	43
	-	2*	33	35
	Total	45	33	78
	RSV A/B - Xpert® Xpress Flu/RSV (Cepheid) and AusDiagnostics Respiratory assays			
		+	**-**	**Total**
	+	13	0	13
	-	1*	64	65
	Total	14	64	78
	SARS-CoV-2 - Panther Fusion® SARS-CoV-2 Assay and VIASURE *SARS-CoV-2* Real Time PCR detection kit			
		+	**-**	**Total**
	+	18	0	18
	-	1#	59	60
	Total	19	59	78

Table 23. Comparative results obtained with 78 clinical samples using different molecular diagnostic kits.

** The low amount of template RNA in these respiratory samples is below the detection limit of the method used. The leftover samples were storage at -20°C, therefore viral RNA degradation could not be discarded due to freeze–thaw cycles.*

 # This sample was analysed again with reference method and confirmed as negative. Therefore, this sample is false positive on Panther Fusion® SARS-CoV-2 Assay.

	VIASURE values vs Reference molecular methods		
Microorganism	**PPA (%)**	**NPA (%)**	**OPA (%)**
SARS-CoV-2	94.74 (73.52 to >99.99)	100 (92.69 to 100)	98.72 (92.41 to >99.99)
Flu A/B	95.56 (84.35 to 99.59)	100 (87.61 to 100)	97.44 (90.58 to 99.84)
RSV A/B	92.86 (66.46 to >99.99)	100 (93.22 to 100)	98.72 (92.41 to >99.99)

Table 24. PPA, NPA and OPA values for VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR detection kit (95% confidence interval).

Results show high agreement to detect SARS-CoV-2, Influenza A/B and RSV A/B using VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit.

8 EXTERNAL QUALITY ASSESSMENT PROGRAMS

CerTest Biotect usually participates in the external quality assessment (EQA) programs of six independent international organizations: **UK NEQAS for Microbiology** (UK NEQAS External Quality Assessment Services), QCMD (Quality Control for Molecular Diagnostics), **INSTAND e.V** (Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V.), **RCPAQAP** (The Royal College of Pathologists of Australasia - Quality Assurance Programs), **CAP** (College of American Pathologists), **LGC Standards Proficiency Testing** and **LABQUALITY**. This allows us to evaluate our laboratories ability to correctly use molecular diagnostic technologies and to verify the quality of our VIASURE products.

UK NEQAS for Microbiology and QCMD are accredited to the EN ISO/IEC 17043:2010 Standards (Conformity assessment - General requirements for proficiency testing) by UKAS (UK's National Accreditation). All panel components from QCMD were produced and certified according to the guidelines for the production and certification of BCR reference materials (EU document BCR/48/93). All EQAs offered by INSTAND e.V. are accredited in accordance with DIN EN ISO/IEC 17043:2010. The RCPAQAP and CAP are accredited for compliance with ISO/IEC 17043, as well. The LGC Standards Proficiency Testing and LABQUALITY are accredited for compliance with ISO/IEC 17043:2010, and ISO 9001:2015.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit will be evaluated with these EQA programmes. The EQAs sample materials used in these programs are intended to mimic the situation of clinical sample processing, and for this reason, samples may contain target organisms in a background of human cells and other components. Therefore, depending on the composition of the corresponding samples, the internal control may or may not be amplified because this qPCR assay uses a human housekeeping gene as an **endogenous IC** (human *RNase P gene*) that is expected to be present in all nucleated human cells.

The performance of VIASURE assay to detect SARS-CoV-2, Flu & RSV viruses has been assessed with several ***INSTAND panels***: **INSTAND Virus Genome Detection – June 2016, June and December 2017, June and November 2018, and June and November 2019, and June 2020 "Respiratory Syncytial Virus"** (nº 359) and **INSTAND Virus Genome Detection – March 2016, May and December 2017, March and November 2018, and March and November 2019, and March 2020 "Influenza A and B Viruses"** (nº 370). (Please, you should consider that we had not officially participated in these programs these years). The results were compared with those obtained by EQA programme final reports.

The **INSTAND EQA Program 2016 "Virus (Genome/Antigen) - Respiratory Syncytial Virus panel** consists of 4 clinical specimens (lyophilized cell lysates) that have been reconstituted in Water RNAse/DNAse free in accordance to the instructions of the supplier. Afterwards, the panel of specimens was extracted with VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch TA150018, expiry date 2017-03, CerTest) and

Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch n°104746, expiry date 2018-05), using the Maxwell® 16 instrument (Promega) and analyzed with VIASURE assay (Batch n° CFR1XL-EXP.469E, expiry date 2022-07). All samples could be detected correctly, and the results are shown in Table 25. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
INSTAND EQA Program 2016 "Virus Genome Detection - Respiratory Syncytial Virus"				
Sample code	**Virus type**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
359029	Respiratory Syncytial Virus A	Negative	Negative	Positive
359030	Respiratory Syncytial Virus A	Negative	Negative	Positive
359031	Negative	Negative	Negative	Negative
359032	Respiratory Syncytial Virus B	Negative	Negative	Positive

Table 25. INSTAND EQA Program June 2016 "Virus Genome Detection - Respiratory Syncytial Virus" results.

The **INSTAND RSV June and December 2017 panel** consisted of 8 lyophilized cell lysates that were reconstituted in RNAse/DNAse free water according to the supplier's instructions. Afterwards, the panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch TA160012 and TA170005, Expiry date 2017-08 and 2018-08, CerTest) and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469E, expiry date 2022-07). All samples could be detected correctly and the results are shown in Table 26. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
INSTAND EQA Programs June and December 2017 "Virus Genome Detection - Respiratory Syncytial Virus"				
Sample code	**Virus type**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
359037	Respiratory Syncytial Virus B	Negative	Negative	Positive
359038	Respiratory Syncytial Virus A	Not available	Not available	Not available
359039	Respiratory Syncytial Virus A	Negative	Negative	Positive
359040	Respiratory Syncytial Virus A	Negative	Negative	Positive
359041	Respiratory Syncytial Virus A	Negative	Negative	Positive
359042	Negative	Negative	Negative	Negative
359043	Respiratory Syncytial Virus B	Negative	Negative	Positive
359044	Respiratory Syncytial Virus B	Negative	Negative	Positive

Table 26. INSTAND EQA Programs June and December 2017 "Virus Genome Detection – Respiratory Syncytial Virus" results.

The **INSTAND RSV June and November 2018 panel** consisted of 8 lyophilized cell lysates that were reconstituted in RNAse/DNAse free water according to the supplier's instructions. Afterwards, the panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch TA170005, Expiry date 2018-08, CerTest), MagDEA Dx SV kit (Batch nº89M030, expiry date 2019-12), using the magLEAD® 12gC instrument (Precision System Science Co.) and Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch nº109011, expiry date 2022-03), using the Maxwell® 16 instrument (Promega); and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry dates 2022-07 and 2022-07). All samples were detected correctly, and the results are shown in Table 27. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
INSTAND EQA Program June and November 2018 "Virus Genome Detection - Respiratory Syncytial Virus"				
Sample code	**Virus type**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
359045	RSV A	Negative	Negative	Positive
359046	RSV B	Negative	Negative	Positive
359047	RSV A	Negative	Negative	Positive
359048	Negative	Negative	Negative	Negative
359049	Respiratory Syncytial Virus B	Negative	Negative	Positive
359050	Respiratory Syncytial Virus A	Negative	Negative	Positive
359051	Negative	Negative	Negative	Negative
359052	Respiratory Syncytial Virus A	Negative	Negative	Positive

Table 27. INSTAND EQA Program June and November 2018 "Virus Genome Detection – Respiratory Syncytial Virus" results.

The **INSTAND RSV June and November 2019 panel** consisted of 8 lyophilized cell lysates that were reconstituted in RNAse/DNAse free water according to the supplier's instructions. Afterwards, the panel of specimens was extracted with MagDEA Dx SV kit (Batch nº92M010, 98M020, expiry date 2020-03 and 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469E, expiry date 2022-07). All samples were detected correctly, and the results are shown in Table 28. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
INSTAND EQA Program June and November 2019 "Virus Genome Detection - Respiratory Syncytial Virus"				
Sample code	**Virus type**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
359053	RSV A	Negative	Negative	Positive
359054	RSV B	Negative	Negative	Positive
359055	RSV A	Negative	Negative	Positive
359056	Negative	Negative	Negative	Negative
359057	RSV A	Negative	Negative	Positive
359058	Negative	Negative	Negative	Negative
359059	RSV B	Negative	Negative	Positive
359060	RSV A	Negative	Negative	Positive

Table 28. INSTAND EQA Program June and November 2019 "Virus Genome Detection – Respiratory Syncytial Virus" results.

The **INSTAND RSV June 2020 panel** consisted of 4 lyophilized cell lysates that were reconstituted in RNAse/DNAse free water according to the supplier's instructions. Afterwards, the panel of specimens was extracted with MagDEA Dx SV kit (Batch nº 17M020, expiry date 2022-03), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469E, expiry date 2022-07). All samples were detected correctly, and the results are shown in Table 29. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
INSTAND EQA Program June 2020 "Virus Genome Detection - Respiratory Syncytial Virus"				
Sample code	**Virus type**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
359061	Negative	Negative	Negative	Negative
359062	RSV A	Negative	Negative	Positive
359063	RSV B	Negative	Negative	Positive
359064	RSV A	Negative	Negative	Positive

Table 29. INSTAND EQA Program June 2020 "Virus Genome Detection – Respiratory Syncytial Virus" results.

"VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit" was evaluated in our facilities with **INSTAND EQA Program March 2016 "Virus Genome Detection - Influenza A and B Viruses" (nº 370).** This panel consists of 6 clinical specimens (lyophilized cell lysates or lyophilized allantoic fluid of infected embryonated chicken eggs) that have been reconstituted in Water RNAse/DNAse free in accordance to the instructions of the supplier. Afterwards, the panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch TA150018, Expiry date 2017-03, CerTest) and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469E, expiry date 2022-07). All samples could be detected correctly, and the results are shown in Table 30. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit					
INSTAND EQA Program 2016 "Virus Genome Detection - Influenza A and B Viruses"					
Sample code	**Virus type/subtype**	**Strain**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
370071	Seasonal influenza A(H3N2) virus	A/Switzerland/9715293/2013	Negative	Positive	Negative
370072	Negative	-	Not available	Not available	Not available
370073	Seasonal influenza B virus	B/Phuket/3073/2013	Negative	Positive	Negative
370074	Avian Influenza A(H5N8) virus	A/Turkey/Germany R2485+86/2014	Not available	Not available	Not available
370075	Seasonal influenza B virus	B/Brisbane/60/2008	Negative	Positive	Negative
370076	Influenza A(H1N1) pdm09- virus	A/California/7/2009/	Negative	Positive	Negative

Table 30. INSTAND EQA Program March 2016 "Virus Genome Detection - Influenza A and B Viruses" results.

The **INSTAND Influenza May and December 2017 panel** consists of 12 lyophilized cell lysates or lyophilized allantoic fluid of infected embryonated chicken eggs that were reconstituted in RNAse/DNAse free water according to the supplier's instructions. Afterwards, the panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch TA160012 and TA170005, Expiry date 2017-08 and 2018-08, CerTest) and Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch nº120981, expiry date 2018-08), using the Maxwell® 16 instrument (Promega); and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry dates 2022-07 and 2022-07). All samples could be detected correctly, and the results are shown in Table 31. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit for BD MAX™					
INSTAND EQA Program May and December 2017 "Virus Genome Detection - Influenza A and B Viruses"					
Sample code	**Virus type/subtype**	**Strain**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
370083	Seasonal influenza A (H3N2)virus	A/Thüringen/5/17	Negative	Positive	Negative
370084	Avian influenza A(H5N8) virus	A/DE-SH/Reiherente/AR8444/ 2013	Not available	Not available	Not available
370085	Avian influenza A(H7N9) virus	A/Anhui/1/2013	Negative	Positive	Negative
370086	Seasonal influenza A(H3N2) virus	A/Switzerland/9715293/ 2013 (vaccine strain)	Negative	Positive	Negative
370087	Seasonal influenza A(H1N1) pdm09 virus	A/Michigian/45/2015 (vaccine strain)	Negative	Positive	Negative
370088	Seasonal influenza B virus	B/Phuket/3073/2013 (vaccine strain)	Negative	Positive	Negative
370089	Negative	Negative	Negative	Negative	Negative
370090	Seasonal influenza A(H1N1) pdm09 virus	A/Michigian/45/2015 (vaccine strain)	Negative	Positive	Negative
370091	Seasonal influenza A (H3N2)virus	A/Thüringen/5/17	Negative	Positive	Negative
370092	Seasonal influenza B virus	B/Phuket/3073/2013 (vaccine strain)	Negative	Positive	Negative
370093	Avian influenza A(H5N8) virus	A/DE-SH/Reiherente/AR8444/ 2013	Negative	Positive	Negative
370094	Avian influenza A(H7N9) virus	A/Anhui/1/2013	Negative	Positive	Negative

Table 31. INSTAND EQA Program May and December 2017 "Virus Genome Detection – Influenza A and B Viruses" results.

The **INSTAND Influenza March and November 2018, March and November 2019 panel** consists of 24 lyophilized cell lysates or lyophilized allantoic fluid of infected embryonated chicken eggs that were reconstituted in RNAse/DNAse free water according to the supplier's instructions. Afterwards, the panel of specimens was extracted with Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch n°109011 and 109347, expiry date 2022-03 and 2022-04), using the Maxwell® 16 instrument (Promega) and MagDEA Dx SV kit (Batch n°89M030 and 98M020, expiry date 2019-12 and 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.), and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry dates 2022-07 and 2022-07). All samples could be detected correctly, and the results are shown in Table 32 and 33. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection					
INSTAND EQA Program March and November 2018 "Virus Genome Detection - Influenza A and B Viruses"					
Sample code	**Virus type/subtype**	**Strain**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
370095	Seasonal influenza A(H1N1) pdm09 virus	A/Michigian/45/2015 (vaccine strain)	Negative	Positive	Negative
370096	Avian influenza A(H7N9) virus	A/Anhui/1/2013	Negative	Positive	Negative
370097	Avian influenza A(H5N8) virus	A/DE-SH/Reiherente/AR8444/ 2016	Negative	Positive	Negative
370098	Seasonal influenza B virus	B/Phuket/3073/2013 (vaccine strain)	Negative	Positive	Negative
370099	Negative	-	Negative	Negative	Negative
370100	Seasonal influenza A(H3N2) virus	A/Thüringen/5/2017	Negative	Positive	Negative
370101	Seasonal influenza B virus	B/Phuket/3073/2013	Negative	Positive	Negative
370102	Negative	-	Negative	Negative	Negative
370103	Seasonal influenza A (H3N2) virus	A/Singapore/INFIMH-16-0019/2016	Negative	Positive	Negative
370104	Influenza A(H1N1) pdm09- virus	A/Michigan/45/2015	Negative	Positive	Negative
370105	Seasonal influenza B virus	B/Colorado/06/2017	Negative	Positive	Negative
370106	Avian influenza A(H5N8) virus	A/DE-SH/Reiherente/AR8444/ 2016	Negative	Positive	Negative

Table 32. INSTAND EQA Program March and November 2018 "Virus Genome Detection – Influenza A and B Viruses" results.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit					
INSTAND EQA Program March and November 2019 "Virus Genome Detection - Influenza A and B Viruses"					
Sample code	**Virus type/subtype**	**Strain**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
370107	Avian influenza A(H5N8) virus	A/DE-SH/Reiherente/AR8444/ 2013	Negative	Positive	Negative
370108	Seasonal influenza B virus	B/Colorado/06/2017	Negative	Positive	Negative
370109	Seasonal influenza A(H1N1) pdm09 virus	A/Michigan/45/2015 (vaccine strain)	Negative	Positive	Negative
370110	Negative	-	Negative	Negative	Negative
370111	Seasonal influenza A (H3N2) virus	A/Singapore/INFIMH-16-0019/2016	Negative	Positive	Negative
370112	Seasonal influenza B virus	B/Phuket/3073/2013 (vaccine strain)	Negative	Positive	Negative
370113	Seasonal influenza A(H1N1)pdm09 virus	A/Brisbane/02/2018 (H1N1)pdm09-like (vaccine strain)	Negative	Positive	Negative
370114	Seasonal influenza B virus	B/Phuket/3073/2013-like (B/Yamagata-line) (vaccine strain)	Negative	Positive	Negative
370115	Negative	-	Negative	Negative	Negative
370116	Avian influenza A(H5N1) virus	A/Whooper Swan/R65/2006 (H5N1)	Negative	Positive	Negative
370117	Seasonal influenza B virus	B/Colorado/06/2017-like (B/Victoria-line) (vaccine strain)	Negative	Positive	Negative
370118	Seasonal influenza A(H3N2) virus	A/Kansas/14/2017 (H3N2)-like (vaccine strain)	Negative	Positive	Negative

Table 33. INSTAND EQA Program March and November 2019 "Virus Genome Detection – Influenza A and B Viruses" results.

The **INSTAND Influenza March 2020 panel** consists of 7 lyophilized cell lysates or lyophilized allantoic fluid of infected embryonated chicken eggs that were reconstituted in RNAse/DNAse free water according to the supplier's instructions. Afterwards, the panel of specimens was extracted MagDEA Dx SV kit (Batch n°98M030, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.), and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469E, expiry dates 2022-07). All samples could be detected correctly, and the results is shown in Table 34. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit					
INSTAND EQA Program March 2020 "Virus Genome Detection - Influenza A and B Viruses"					
Sample code	**Virus type/subtype**	**Strain**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
370119	seasonal influenza B virus	B/Colorado/06/2017-like (B/Victoria-line) (vaccine strain)	Negative	Positive	Negative
370120	seasonal influenza A(H1N1)pdm09 virus	A/Brisbane/02/2018 (H1N1)pdm09-like (vaccine strain)	Negative	Positive	Negative
370121	seasonal influenza B virus	B/Phuket/3073/2013-like (B/Yamagata-line) (vaccine strain)	Negative	Positive	Negative
370122	Negative	-	Negative	Negative	Negative
370123	seasonal influenza A(H3N2) virus	A/Kansas/14/2017 (H3N2)-like (vaccine strain)	Negative	Negative	Negative
370124	avian influenza A(H5N1) virus	A/Whooper Swan/R65/2006 (H5N1)	Negative	Positive	Negative
370125	avian influenza A(H5N8) virus	A/DE-SH/Reiherente/AR 8444/2016 (H5N8)	Negative	Positive	Negative

Table 34. INSTAND EQA Program March 2020 "Virus Genome Detection – Influenza A and B Viruses" results.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit has been assessed with ***QCMD Programs***: **QCMD 2016, 2017 Respiratory Syncytial Virus RNA EQA Programme, QCMD 2017 Influenza virus (RNA) EQA Programme, QCMD 2017 Respiratory I Pilot Study EQA Panel, QCMD 2018 Influenza virus A and B (RNA) EQA Programme**, **QCMD 2017, 2018 and 2019 Influenza Haemagglutinin Typing EQA Programme** (Please, you should consider that we had not officially participated in these programs these years). The results were compared with those obtained by EQA programme final reports.

The **QCMD 2016 Respiratory Syncytial Virus RNA EQA Programme** consist of 8 vials containing frozen transport medium samples with various concentrations of respiratory syncytial virus or samples negative for respiratory syncytial virus. The panel of specimens was extracted with Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch n°104746, expiry date 2018-05), using the Maxwell® 16 instrument (Promega) and RIDA® Xtract (Batch n°QL150012, expiry date 2017-04, R-Biopharm®) and analyzed with VIASURE assay (Batch n° CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry dates 2022-07 and 2022-07). All samples could be detected correctly, and the results are shown in Table 35. Please, you should consider that we

had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2016 Respiratory Syncytial Virus RNA EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
RSV16S - 01	RSV Type A	Not available	Not available	Not available
RSV16S -02	RSV Type A	Not available	Not available	Not available
RSV16S -03	RSV Type A	Not available	Not available	Not available
RSV16S -04	Negative	Negative	Negative	Negative
RSV16S -05	RSV Type A	Negative	Negative	Positive
RSV16S -06	RSV Type B	Not available	Not available	Not available
RSV16S -07	RSV Type B	Negative	Negative	Positive
RSV16S -08	Metapneumovirus Type A2	Negative	Negative	Negative

Table 35. QCMD 2016 Respiratory Syncytial Virus (RNA) EQA Programme results.

The **QCMD 2017 Respiratory Syncytial Virus (RNA) EQA Programme** consist of 10 vials containing frozen transport medium samples with various concentrations of respiratory syncytial virus or samples negative for respiratory syncytial virus. The **QCMD 2017 Influenza virus A and B (RNA) EQA Programme** consist of 10 frozen transport medium samples with various concentrations of Influenza A or B or samples negative for Influenza A or B. The panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch TA170005, Expiry date 2018-08, CerTest), Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch n°109347, expiry date 2022-04), using the Maxwell® 16 instrument (Promega), and MagDEA Dx SV kit (Batch n°89M030, 89M040 and 18M010, expiry date 2019-12, 2019-12 and 2022-04), using the magLEAD® 6gC instrument (Precision System Science Co.); and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469C, CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry dates 2022-07, 2022-07 and 2022-07). All samples could be detected correctly and the results are shown in Tables 36 and 37. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2017 Respiratory Syncytial Virus RNA EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
RSV17S - 01	RSV Type B	Negative	Negative	Positive
RSV17S -02	RSV Type A	Negative	Negative	Positive
RSV17S -03	Negative	Negative	Negative	Negative
RSV17S -04	RSV Type B	Negative	Negative	Positive
RSV17S -05	RSV Type B	Negative	Negative	Positive
RSV17S -06	RSV Type A	Not available	Not available	Not available
RSV17S -07	RSV Type B	Negative	Negative	Positive
RSV17S -08	RSV Type A	Negative	Negative	Positive
RSV17S -09	RSV Type B	Negative	Negative	Positive
RSV17S -10	RSV Type A	Negative	Negative	Positive

Table 36. QCMD 2017 Respiratory Syncytial Virus (RNA) EQA Programme results.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2017 Influenza virus A and B (RNA) EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
INFRNA17S- 01	Influenza virus A (H1N1)	Negative	Positive	Negative
INFRNA17S -02	Influenza virus B (Victoria)	Negative	Positive	Negative
INFRNA17S -03	Influenza virus B (Victoria)	Negative	Positive	Negative
INFRNA17S -04	Influenza A and B Negative	Negative	Negative	Negative
INFRNA17S -05	Influenza virus A (H1N1)	Negative	Positive	Negative
INFRNA17S -06	Influenza virus A (H3N2)	Negative	Positive	Negative
INFRNA17S -07	Influenza virus B (Yamagata)	Negative	Positive	Negative
INFRNA17S -08	Influenza virus A (H3N2)	Negative	Positive	Negative
INFRNA17S -09	Influenza virus A (H1N1 pdm09)	Negative	Positive	Negative
INFRNA17S -10	Influenza virus B (Victoria)	Negative	Positive	Negative

Table 37. QCMD 2017 Influenza virus A and B (RNA) EQA Programme results.

The **QCMD 2017 Respiratory I Pilot Study EQA Panel** consist of 10 frozen transport medium samples with various concentrations of influenza virus A or B or respiratory syncytial virus or samples negative for both influenza virus A & B and respiratory syncytial virus. The **QCMD 2017 Influenza Haemagglutinin typing EQA Panel** consists of 8 frozen transport medium samples with various concentrations and types of influenza virus or samples negative for influenza virus (Please, you should consider that we had not participated officially that year). The panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref.

VS-KE06, Batch nº TA160012 and TA170005, expiry date 2017-08 and 2018-08, CerTest); and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469E, expiry date 2022-07). The results were compared with those presented by the EQA's programme final report. All samples could be detected correctly and the results are shown in Tables 38 and 39. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2017 Respiratory I EQA Pilot Study RNA EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
RESPI17S-01	Influenza Type B (Victoria)	Negative	Positive	Negative
RESPI17S-02	Influenza Type A (H1N1)	Negative	Positive	Negative
RESPI17S-03	Influenza Type A (H7N7)	Negative	Positive	Negative
RESPI17S-04	Influenza Type B (Yamagata)	Negative	Positive	Negative
RESPI17S-05	Influenza Type A (H5N1)	Not available	Not available	Not available
RESPI17S-06	Negative	Negative	Negative	Negative
RESPI17S-07	RSV Type B	Negative	Negative	Positive
RESPI17S-08	RSV Type B	Negative	Negative	Positive
RESPI17S-09	RSV Type A	Negative	Negative	Positive
RESPI17S-10	RSV Type A	Negative	Negative	Positive

Table 38. QCMD 2017 Respiratory I EQA Pilot Study RNA EQA Programme results.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2017 Influenza Haemagglutinin Typing EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
INFHT17S - 01	Influenza virus H1N1 pdm09	Negative	Positive	Negative
INFHT17S -02	Influenza virus H7N7	Negative	Positive	Negative
INFHT17S -03	Influzenza virus H3N2	Negative	Positive	Negative
INFHT17S -04	Influenza virus H1N1 pdm09	Negative	Positive	Negative
INFHT17S -05	Influzenza virus H3N2	Negative	Positive	Negative
INFHT17S -06	Influenza virus H5N1	Negative	Positive	Negative
INFHT17S -07	Influenza virus negative	Negative	Negative	Negative
INFHT17S -08	Influenza virus H5N1	Negative	Positive	Negative

Table 39. QCMD 2017 Influenza Haemagglutinin Typing EQA Programme results.

The **QCMD 2018 Influenza virus A and B (RNA) EQA Programme** consist of 10 frozen transport medium samples with various concentrations of Influenza A or B or samples negative for Influenza A or B. The panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch nº

TA170005, expiry date 2018-08, CerTest) and MagDEA Dx SV kit (Batch n°18M010, expiry date 2022-04), using the magLEAD® 12gC instrument (Precision System Science Co.); and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469C and CFR1XL-EXP.469E, expiry dates 2022-07 and 2022-07). All samples could be detected correctly, and the results are shown in Tables 40. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2018 Influenza virus A and B (RNA) EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
INFRNA18S- 01	Influenza virus A (H1N1 pdm09)	Negative	Positive	Negative
INFRNA18S -02	Influenza virus A (H1N1 pdm09)	Negative	Positive	Negative
INFRNA18S -03	Influenza virus negative	Negative	Negative	Negative
INFRNA18S -04	Influenza virus B (Yamagata)	Negative	Positive	Negative
INFRNA18S -05	Influenza virus B (Yamagata)	Negative	Negative*	Negative
INFRNA18S -06	Influenza virus B (Victoria)	Negative	Positive	Negative
INFRNA18S -07	Influenza virus B (Victoria)	Negative	Positive	Negative
INFRNA18S -08	Influenza virus A (H3N2)	Negative	Positive	Negative
INFRNA18S -09	Influenza virus A (H3N2)	Negative	Positive	Negative
INFRNA18S -10	Influenza virus A (H3N2)	Negative	Positive	Negative

Table 40. QCMD 2018 Influenza virus A and B (RNA) EQA Programme results. *Eluate negative for Influenza B.

The **QCMD 2018 Influenza Haemagglutinin typing EQA Panel** consists of 8 frozen transport medium samples with various concentrations and types of influenza virus or samples negative for influenza virus. The panel of specimens was extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, Batch n° TA170005, expiry date 2018-08, CerTest) Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch n°109347, expiry date 2022-04), using the Maxwell® 16 instrument (Promega), and MagDEA Dx SV kit (Batch n°18M010, expiry date 2022-04), using the magLEAD® 12gC instrument (Precision System Science Co.); and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469C, CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry dates 2022-07, 2022-07 and 2022-07). All samples could be detected correctly, and the results are shown in Table 41. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2018 Influenza Haemagglutinin Typing EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
INFHT18S - 01	Influenza virus A (H5N1)	Negative	Positive	Negative
INFHT18S -02	Influzenza virus A (H3N2)	Negative	Positive	Negative
INFHT18S -03	Influenza virus negative	Negative	Negative	Negative
INFHT18S -04	Influzenza virus A (H3N2)	Negative	Positive	Negative
INFHT18S -05	Influzenza virus A (H3N2)	Negative	Positive	Negative
INFHT18S -06	Influzenza virus A (H7N7)	Negative	Positive	Negative
INFHT18S -07	Influzenza virus A (H7N7)	Negative	Positive	Negative
INFHT18S -08	Influenza Virus A (H1N1 pdm09)	Negative	Positive	Negative

Table 41. QCMD 2018 Influenza Haemagglutinin Typing EQA Programme results.

The **QCMD 2019 Influenza Haemagglutinin typing EQA Panel** consists of 8 frozen transport medium samples with various concentrations and types of influenza virus or samples negative for influenza virus. The panel of specimens was extracted with with MagDEA Dx SV kit (Batch nº 98M020, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.); and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469E, expiry date 2022-07). All samples could be detected correctly, and the results are shown in Table 42. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit				
QCMD 2019 Influenza Haemagglutinin Typing EQA Programme				
Sample code	**Sample content**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
INFHT19S - 01	Influenza virus A (H5N1)	Negative	Positive	Negative
INFHT19S -02	Influzenza virus A (H3N2)	Negative	Positive	Negative
INFHT19S -03	Influenza virus negative	Negative	Negative	Negative
INFHT19S -04	Influzenza virus A (H7N7)	Negative	Positive	Negative
INFHT19S -05	Influenza Virus A (H1N1 pdm09)	Negative	Positive	Negative
INFHT19S -06	Influenza virus A (H5N1)	Negative	Positive	Negative
INFHT19S -07	Influzenza virus A (H3N2)	Negative	Positive	Negative
INFHT19S -08	Influzenza virus A (H3N2)	Negative	Positive	Negative

Table 42. QCMD 2019 Influenza Haemagglutinin Typing EQA Programme results.

VIASURE kit was evaluated in our facilities with the **NEQAS for Microbiology**, **"Molecular detection of respiratory viruses program 2017-2018"** (May 2017 - Distribution: 4095, October 2017 - Distribution: 4167, January 2018 - Distribution: 4227). Each panel consisted of Simulated nasopharyngeal swab (3), Simulated nasopharyngeal aspirate (6), and Simulated throat swab (3). The panels of specimens were extracted with "VIASURE RNA-DNA Extraction kit" (Ref. VS-KE06, TA160012 and TA170005, expiry dates 2017-08 and 2018-08, CerTest) and Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Batch n°109347, expiry date 2022-04), using the Maxwell® 16 instrument (Promega); and analysed with VIASURE assay (Batch CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry dates 2022-07 and 2022-07). All samples could be detected correctly, and the results are shown in Table 43. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
NEQAS for Microbiology 2017/2018 - Molecular detection of respiratory viruses' program				
May 2017 - Distribution: 4095				
Sample code	**Sample composition**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
3813	Influenza virus A (H3N2)	Negative	Positive	Negative
3814	Influenza virus B	Negative	Positive	Negative
3815	Respiratory Syncytial Virus	Negative	Negative	Positive
3816	Parainfluenza	Negative	Negative	Negative
October 2017 - Distribution: 4167				
Sample code	**Sample composition**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
4028	Influenza type A (H1N1)	Negative	Positive	Negative
4029	Influenza type B	Negative	Positive	Negative
4030	Influenza type A (H3N2)	Negative	Positive	Negative
4031	Rhinovirus type 2	Negative	Negative	Negative
January 2018 - Distribution: 4227				

Sample code	Sample composition	Viasure – SARS-CoV-2	Viasure - Flu	Viasure - RSV
4217	Influenza virus A H1N1	Negative	Positive	Negative
4218	Influenza virus B	Negative	Positive	Negative
4219	Influenza virus A H3N2	Negative	Positive	Negative
4220	Adenovirus Type 2	Negative	Negative	Negative

Table 43. NEQAS Program 2017-2018 "Molecular detection of respiratory viruses" results.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit, has been evaluated in our facilities with **RCPAQAP Survey MAV1:2019, MAV2:2019 and MAV3:2019 Molecular Influenza Programme**. These panels consist of 18 samples of inactivated virus from human and animal strains of influenza virus suspended in 500uL phosphate buffered gelatin saline. The panels of specimens were extracted with MagDEA Dx SV kit (Batch n°92M010, 94M010, 17M020; expiry date 2020-03, 2020-05 and 2022-03), using the magLEAD® 12gC instrument (Precision System Science Co.); and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469E, expiry date 2022-07). The results were compared with those presented by the EQA's programme final reports. All samples could be detected correctly, the results are shown in Table 44 (Please, you should consider that we had not officially participated in Survey MAV1:2019).

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
RCPA Survey MAV1:2019 Molecular Influenza Programme				
Sample code	**Virus Strain**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
1A	A/Brisbane/117/2018 H3N2	Negative	Positive	Negative
1B	A/Sydney/134/2018 A/(H1N1)pdm	Negative	Positive	Negative
1C	MDCK	Negative	Negative	Negative
1D	B/Maryland/15/2016	Negative	Positive	Negative
1E	A/Chicken/Myanmar/433/2016 H9N2	Negative	Positive	Negative
1F	A/Brisbane/1028/2017 H3N2	Negative	Positive	Negative
RCPA Survey MAV2:2019 Molecular Influenza Programme				
Sample code	**Virus Strain**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
2A	A/Guangdong/17SF003/2016 A/H7N9	Negative	Positive	Negative
2B	A/Brisbane/1028/2017 A/H3N2	Negative	Positive	Negative
2C	B/Townsville/8/2016 (Victoria lineage)	Negative	Positive	Negative
2D	MDCK cells	Negative	Negative	Negative
2E	A/Perth/1001/2018 A/H3N2	Negative	Positive	Negative
2F	A/Victoria/2040/2018 A/H1N1pdm	Negative	Positive	Negative

RCPA Survey MAV3:2019 Molecular Influenza Programme				
Sample code	**Virus Strain**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
3A	B/Canberra/11/2016 (Yamagata lineage)	Negative	Positive	Negative
3B	A/Victoria/2040/2018 A/H1N1pdm	Negative	Positive	Negative
3C	A/Perth/1001/2018 A/H3N2	Negative	Positive	Negative
3D	A/Duck/Lao/XBY004/2014 A/H5N6 (Clade 2.3.4.4)	Negative	Positive	Negative
3E	A/Sydney/134/2018 A/H1N1pdm	Negative	Positive	Negative
3F	A/Brisbane/117/2018 A/H3N2	Negative	Positive	Negative

Table 44. RCPA Surveys MAV1:2019, MAV2:2019 and MAV3:2019 Molecular Influenza Programme results.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit, has been evaluated in our facilities with **RCPAQAP Survey MI-MAV-20-07/12 Molecular Influenza Programme**. These panels consist of 6 samples of inactivated virus from human and animal strains of influenza virus suspended in 500uL phosphate buffered gelatin saline. The panels of specimens were extracted with MagDEA Dx SV kit (Batch n°17M020, expiry date 2022-03), using the magLEAD® 12gC instrument (Precision System Science Co.); and analysed with VIASURE assay (Batch n° CFR1XL-EXP.469E, expiry date 2022-07). The results were compared with those presented by the EQA's programme final reports. All samples could be detected correctly, the results are shown in Table 45 (Please, you should consider that we had not officially participated in Survey MAV1:2019).

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
RCPA Survey MAV1:2019 Molecular Influenza Programme				
Sample code	**Virus Strain**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
20-07	A/Perth/1001/2018 A/H3N2	Negative	Positive	Negative
20-08	MDCK	Negative	Negative	Negative
20-09	A/Sydney/134/2018 A/H1N1pdm	Negative	Positive	Negative
20-10	B/Canberra/11/2016 (Yamagata lineage)	Negative	Positive	Negative
20-11	A/Duck/Lao/XBY004/2014 A/H5N6 (Clade 2.3.4.4)	Negative	Positive	Negative
20-12	A/Newcastle/607/2019 A/H3N2	Negative	Positive	Negative

Table 45. RCPA Surveys MI-MAV-20-07/12 Molecular Influenza Programme results.

Finally, the VIASURE kit was evaluated in our facilities with **CAP Influenza A, Influenza B, RSV by NAA, Surveys: ID3-A 2019, ID3-B 2019 and ID3-C 2020.** These panels consist of 15 simulated body fluids. The panels of specimens were extracted with MagDEA Dx SV kit (Batch n° 94M010, 98M020; expiry date 2020-05, 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.); and analysed with

VIASURE assay (Batch nº CFR1XL-EXP.469D and CFR1XL-EXP.469E, expiry date 2022-07). The results were compared with those presented by the EQA's programme final reports. All samples could be detected correctly, and the results are shown in Table 46 (Please, you should consider that we had not officially participated in any Survey).

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
CAP Survey ID3-A:2019				
Sample code	**Virus Strain**	**Viasure - SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
ID3-01	Influenza B/Massachusetts/2/2012	Negative	Positive	Negative
ID3-02	Negative	Negative	Negative	Negative
ID3-03	RSV Type B CH93(18)-18	Negative	Negative	Positive
ID3-04	Influenza A/New York/18/2009 (H1N1)(pdm)	Negative	Positive	Negative
ID3-05	Influenza B/Brisbane/60/2008	Negative	Positive	Negative
CAP Survey ID3-B:2019				
Sample code	**Virus Strain**	**Viasure - SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
ID3-06	Influenza A/Texas/50/2012 (H3N2)	Negative	Positive	Negative
ID3-07	Negative	Negative	Negative	Negative
ID3-08	Influenza A/Michigan/45/2015 (H1N1)(pdm)	Negative	Positive	Negative
ID3-09	RSV Type A	Negative	Negative	Positive
ID3-10	RSV Type B/CH93(18)-18	Negative	Negative	Positive
CAP Survey ID3-C:2019				
Sample code	**Virus Strain**	**Viasure - SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
ID3-11	B/Phuket/3073/2013 (B/Yamagata lineage)	Negative	Positive	Negative
ID3-12	RSV	Negative	Negative	Positive
ID3-13	RSV Type A	Negative	Negative	Positive
ID3-14	Negative	Negative	Negative	Negative
ID3-15	A/Hong Kong/4801/2014 (H3N2)	Negative	Positive	Negative

Table 46. CAP Influenza A, Influenza B, RSV by NAA (ID3-A 2019, ID3-B 2019 and ID3-C 2019) Programme results.

Finally, the VIASURE kit was evaluated in our facilities with **CAP Influenza A, Influenza B, RSV by NAA, Surveys: ID3-A 2020.** These panels consist of 5 simulated body fluids. The panels of specimens were extracted with MagDEA Dx SV kit (Batch nº 98M030; expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.); and analysed with VIASURE assay (Batch nº CFR1XL-EXP.469E, expiry date 2022-07). The results were compared with those presented by the EQA's programme final reports. All samples could be detected correctly, and the results are shown in Table 47 (Please, you should consider that we had not officially participated in Survey ID3-A 2020).

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
CAP Survey ID3-A:2020				
Sample code	**Virus Strain**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
ID3-01	Influenza A, strain Michigan/45/2015 (H1N1)(pdm09)	Negative	Positive	Negative
ID3-02	RSV Type A	Negative	Negative	Positive
ID3-03	Influenza B, strain Phuket/3073/2013 (Yamagate Lineage)	Negative	Positive	Negative
ID3-04	RSV Type B/CH93 (18)-18	Negative	Negative	Positive
ID3-05	Negative	Negative	Negative	Negative

Table 47. CAP Influenza A, Influenza B, RSV by NAA (ID3-A 2020) Programme results.

The performance of **VIASURE assay to detect SARS-CoV-2** has been analyzed by testing the ***INSTAND, QCMD, NEQAS, CAP, LGC and LABQUALITY new programs and panels.***

The **INSTAND EQA Program April 2020 "Virus Genome Detection – SARS-CoV-2 panel** consists of 7 lyophilized specimens (derive from lysates of cells which have been infected with coronavirus (SARS-CoV-2, HCoV OC43 or HCoV 229E)) that have been reconstituted in Water RNAse/DNAse free in accordance to the instructions of the supplier. Samples positive for SARS-CoV-2 contain heat inactivated virus. Negative samples derive from lysates of non-infected cells.

The EQA sample panel was extracted with MagDEA Dx SV kit, using the magLEAD® 12gC instrument (Precision System Science Co.) (Batch nº 98M030, Expiry date 2020-09). Afterwards, the RNA samples were analyzed with VIASURE assay (Batch nº CFR1XL-EXP.469E, expiry date 2022-07) on AriaMx Realtime PCR System (Agilent Technologies) and an additional competitor test (Vircell SARS-CoV-2 Real Time PCR Detection Kit, which detect *N* gene of nCoV and *E* gene of SARS-related coronaviruses, Reference nº RTPCR001, expiry date 2021-08). All samples could be detected correctly, and the results are shown in Table 48. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
INSTAND EQA Program April 2020 "Virus Genome Detection – SARS-CoV-2 panel"				
Sample code	**Sample source**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
340059	SARS-CoV-2 positive	Positive	Negative	Negative
340060	SARS-CoV-2 negative (HCoV OC43 positive)	Negative	Negative	Negative
340061	SARS-CoV-2 positive	Positive	Negative	Negative
340062	SARS-CoV-2 negative	Negative	Negative	Negative
340063	SARS-CoV-2 positive	Positive	Negative	Negative
340064	SARS-CoV-2 positive	Positive	Negative	Negative
340065	SARS-CoV-2 negative (HCoV 229E positive)	Negative	Negative	Negative

Table 48. INSTAND EQA Program April 2020 "Virus Genome Detection – SARS-CoV-2 panel".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit was evaluated with **QCMD 2020 Coronavirus Outbreak Preparedness EQA Pilot Study** (CVOP20). The EQA sample panel was extracted with MagDEA Dx SV kit (Batch n°98M030, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit (Batch n° CFR1XL-EXP.469E, expiry date 2022-07) on AriaMx Realtime PCR System (Agilent Technologies). The panel consist of 8 frozen transport medium samples with various concentrations of Coronavirus or samples negative for Coronavirus. The results were compared with those presented by the EQA's programme final report. All samples could be detected correctly, and the results are shown in Table 49. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
QCMD 2020 Coronavirus Outbreak Preparedness EQA Pilot Study				
Sample code	**Sample source**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
CVOP20S-01	SARS-CoV-2 positive	Positive	Negative	Negative
CVOP20S-02	SARS-CoV-2 negative (HCoV NL63 positive)	Negative	Negative	Negative
CVOP20S-03	SARS-CoV-2 positive	Positive	Negative	Negative
CVOP20S-04	SARS-CoV-2 negative (HCoV OC43 positive)	Negative	Negative	Negative
CVOP20S-05	Negative	Negative	Negative	Negative
CVOP20S-06	SARS-CoV-2 positive	Positive	Negative	Negative
CVOP20S-07	SARS-CoV-2 positive	Positive	Negative	Negative
CVOP20S-08	SARS-CoV-2 positive	Positive	Negative	Negative

Table 49. QCMD 2020 Coronavirus Outbreak Preparedness EQA Pilot Study.

VIASURE kit was evaluated in our facilities with the **NEQAS for Microbiology**, **"Molecular detection of SARS-CoV-2"** (August 2020 - Distribution: 4886). The panels of specimens were extracted with MagDEA Dx SV kit (Batch nº18M020, expiry date 2022-05), using the magLEAD® 12gC instrument (Precision System Science Co.)); and analysed with VIASURE assay (Batch CFR1XL-EXP.469D, expiry date 2022-07). All samples could be detected correctly, and the results are shown in Table 50.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
NEQAS for Microbiology 2020/2021 - Molecular detection of SARS-CoV-2				
August 2020 - Distribution: 4886				
Sample code	**Sample composition**	**Viasure – SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
6333	SARS-CoV-2 negative	Negative	Negative	Negative
6334	SARS-CoV-2 positive	Positive	Negative	Negative

Table 50. NEQAS for Microbiology 2020/2021 - Molecular detection of SARS-CoV-2 results.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit was evaluated with **LGC Standards Proficiency Testing 2020** (COV-SARS-CoV-2 molecular). The EQA sample panels were extracted with MagDEA Dx SV kit (Batch nº98M030, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit (Batch nº CFR1XL-EXP.469E, expiry date 2022-07) on AriaMx Realtime PCR System (Agilent Technologies). The panel consist of 2 samples, one positive for Coronavirus and another negative for Coronavirus. The results were compared with those presented by the EQA's programme final report. All samples could be detected correctly, and the results are shown in Table 51. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
LGC Standards Proficiency Testing 2020. COV-SARS-CoV-2 molecular.				
Sample code	**Sample source**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
COV-A	SARS-CoV-2 negative	Negative	Negative	Negative
COV-B	SARS-CoV-2 positive	Positive	Negative	Negative

Table 51. LGC Standards Proficiency Testing 2020. COV-SARS-CoV-2 molecular.

Finally, the VIASURE kit was evaluated in our facilities with **CAP SARS-CoV-2 Molecular Proficiency Testing Program COV2A-2020.** These panels consist of 3 non-infectious liquid specimens. The EQA sample panels were extracted with MagDEA Dx SV kit (Batch n°98M030, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit (Batch n° CFR1XL-EXP.469E, expiry date 2022-07) on AriaMx Realtime PCR System (Agilent Technologies). The results were compared with those presented by the EQA's programme final reports. All samples could be detected correctly, and the results are shown in Table 52.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
CAP SARS-CoV-2 Molecular Proficiency Testing Program COV2A-2020				
Sample code	**Sample source**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
COV2-01	SARS-CoV-2 positive	Positive	Negative	Negative
COV2-02	SARS-CoV-2 negative	Negative	Negative	Negative
COV2-03	SARS-CoV-2 positive	Positive	Negative	Negative

Table 52. CAP SARS-CoV-2 Molecular Proficiency Testing Program COV2A-2020.

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit was evaluated with **LABQUALITY SARS-CoV-2, nucleic acid detection. Round 1, 2020 (Pilot).** This panel consists of 2 simulated swab samples, which included the whole genome of SARS-CoV-2 virus. The EQA sample panels were extracted with MagDEA Dx SV kit (Batch n°98M030, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit (Batch n° CFR1XL-EXP.469E, expiry date 2022-07) on AriaMx Realtime PCR System (Agilent Technologies). The results were compared with those presented by the EQA's programme final reports. All samples could be detected correctly, and the results are shown in Table 53. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit				
LABQUALITY SARS-CoV-2, nucleic acid detection. Round 1, 2020 (Pilot)				
Sample code	**Sample source**	**Viasure -SARS-CoV-2**	**Viasure - Flu**	**Viasure - RSV**
S003 (LQ775720013)	SARS-CoV-2 negative	Negative	Negative	Negative
S004 (LQ775720014)	SARS-CoV-2 positive	Positive	Negative	Negative

Table 53. LABQUALITY SARS-CoV-2, nucleic acid detection. Round 1, 2020 (Pilot).

Tu sum up, using 2016 to 2020 EQA programmes, **VIASURE assay found 87/224 positive samples for Influenza A, 33/224 positive samples for Influenza B, 47/224 positive samples for RSV and 14/224 SARS-CoV-2.**

The positive percentage agreement (PPA), the negative percentage agreement (NPA) and the overall percentage agreement (OPA) for the pathogen were calculated with 95% confidence interval (CI). The statistical values calculation was based on the *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests* (FDA Guide March 2007). The values were calculated by Microsoft Excel 2010 for Windows and the online resource GraphPad QuickCalcs. The 95% CI were calculated using the modified Wald method. The results are described below in Table 54.

	VIASURE VALUES in EQA programmes from 2016-2020			
	Flu A	**Flu B**	**RSV**	**SARS-CoV-2**
PPA	100% (94.93% to 100%)	97.06% (83.78% to 99.99%)	100% (90.98% to 100%)	100% (74.85% to 100%)
NPA	100% (96.72% to 100%)	100% (97.61% to 100%)	100% (97.44% to 100%)	100% (97.84 to 100%)
OPA	100% (97.97% to 100%)	99.55% (97.26% to 99.99)	100% (97.97% to 100%)	100 (0.9797 to 1.0000)

Table 54. Positive percentage agreement (PPA), negative percentage agreement (NPA) and overall percentage agreement (OPA) for different pathogens with 95% CI.

All these results showed the high agreement to detect SARS-CoV-2 using VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit.

The results of programs that we had not officially participated have been recorded internally on Excel data sheet "4 Clinical performance CFR1". The results of programs that we had participated have been recorded internally on Excel data sheets "EQA Programme Results".

All these results showed the high sensitivity and specificity to detect SARS-CoV-2, influenza and RSV viruses using VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit.

9 EXPECTED VALUES

Bibliographic research of the analysis of the prevalence of SARS-CoV-2 in clinical samples

Respiratory viral infections in humans represent a significant global health burden. A wide variety of viruses can be held responsible for this, one of them being Coronaviruses (CoVs), which is a group of

large enveloped RNA viruses that belong to the *Coronaviridae* family. Six different CoVs strains that infect humans have been identified: CoV-229E, CoV-OC43, CoV-NL63, CoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). In December 2019, some people that worked at or lived around the Huanan seafood market in Wuhan, Hubei Province, China, have presented pneumonia of unknown cause. Deep sequencing analysis of the respiratory samples indicated a novel coronavirus, which was named firstly 2019 novel coronavirus (2019-nCoV) and lately SARS-CoV-2.

SARS-CoV-2 belongs to the genus *Betacoronavirus* but it is enough divergent from SARS-CoV to be considered a new human-infecting coronavirus. Although the pneumonia is the principal illness associated, a few patients have developed severe pneumonia, pulmonary edema, acute respiratory distress syndrome, or multiple organ failure and death. Centers of Disease Control and Prevention (CDC) believes that symptoms of SARS-CoV-2 may appear in as few as 2 days or as long as 14 days after exposure, being the most common fever or chills, cough, myalgia, dyspnea and loss of taste or smell. Less common symptoms are sore throat, headache, diarrhea and vomiting. It seems that people above 60 years old, males, and people with comorbidities most often have severe disease.

The SARS-CoV-2 have been reported in all continents and the case-fatality rate in some countries have reached the 15%, higher than SARS-CoV (10%) and less than MERS-CoV (34%). Due to some patients were linked to a seafood market, it suggested animal-to-human transmission. But, soon, human-to-human transmission of the SARS-CoV-2 has been confirmed, even in the incubation period without symptoms. It has been estimated that the transmission rate is 2-3%, like that of SARS (3%).

Common diagnosis of SARS-CoV-2 in clinical samples

Early and accurate diagnosis of SARS-CoV-2 infection is important for clinical management, including appropriate infection prevention and control measures and optimized supportive care for seriously ill patients.

Currently, any patient who meets the definition of a suspected case of pneumonia associated with a SARS-CoV-2 should be screened for the virus with RT-PCR, which includes i) patient with severe acute respiratory infection (fever and at least one sign or symptom of respiratory disease, for example, cough or shortness of breath), AND a history of travel to or residence in a country, area or territory that has reported local transmission of COVID-19 disease during the 14 days prior to onset of symptoms. OR ii) patient with any acute respiratory illness AND who has been a contact of a confirmed or probable case of COVID-19 disease during the 14 days prior to the onset of symptoms, OR iii) a patient with severe acute respiratory infection (that is, fever and at least one sign or symptom of respiratory disease, for example, cough or shortness breath) AND who requires hospitalization AND who has no other etiology that fully explains the clinical presentation.

The decision to test should be based on clinical and epidemiological factors and linked to an assessment of the likelihood of infection.

CDC recommends upper respiratory tract specimens (nasopharyngeal (NP) swab, oropharyngeal (OP) swabs, nasal mid-turbinate swab, nasal swab, nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) specimens collected mainly by a healthcare provider) and/or lower respiratory specimens (sputum, endotracheal aspirate, or bronchoalveolar lavage in patients with more severe respiratory disease) for the identification of SARS-CoV-2. In addition, other clinical specimens as blood and stool may be collected to monitor the presence of the virus.

Molecular diagnosis of SARS-CoV-2 could be performed using a pan-coronavirus assay for amplification followed by sequencing of amplicons from non-conserved regions for characterization and confirmation, or amplification and detection of SARS-CoV-2 specific sequences by real-time RT-PCR methods. Several assays that detect the SARS-CoV-2 have been are currently available, such as China CDC (gene targets, ORF1ab and N), Charité – Germany (gene targets, RdRP and E) or US CDC (three targets in N gene). Serological testing may be useful to confirm immunologic response to a pathogen from a specific viral group, e.g. coronavirus. Best results from serologic testing requires the collection of paired serum samples (in the acute and convalescent phase) from cases under investigation. On the other hand, regular sequencing of clinical case samples can be useful for monitoring mutations in the viral genome in order to improve the performance of diagnostic tests.

Influenza

Influenza activity in the northern hemisphere continued to decrease with a predominance of influenza B virus reported. In temperate countries in the southern hemisphere, influenza activity started to increase slightly in South America and South Africa, but remained low overall in most of Oceania.

- In North America, influenza activity continued to decrease while the proportion of influenza B virus detections increased compared to previous reporting periods.
- Influenza activity continued to decrease in Europe and temperate Asia with a predominance of influenza B virus activity.
- In Africa, influenza activity was generally low with influenza A virus detections reported in Western Africa and influenza A and B virus detections reported in Eastern Africa.
- In Central America and the Caribbean countries, influenza and other respiratory virus activity remained generally low, although levels of A(H1N1)pdm09 virus activity continued to increase in El Salvador, Guatemala and Panama. Active circulation of influenza A (H1N1)pdm09 was also

reported in Suriname. In Jamaica, severe acute respiratory infection (SARI) activity remained above the threshold and an increase in pneumonia cases was observed.

- In tropical South America, increased influenza A (H1N1)pdm09 activity was reported in Bolivia. SARI activity was elevated in Ecuador together with a high proportion of samples positive for respiratory syncytial virus (RSV) and influenza A(H1N1)pdm09 virus. Peru reported increased RSV detections.
- In tropical countries of South Asia, influenza activity was generally low but several countries reported increased influenza virus detections.
- In temperate South America, influenza-like illness (ILI) activity increased in recent weeks above seasonal thresholds. Increases in RSV activity in the region and influenza A detections in Argentina and Uruguay were also reported.
- In the temperate countries of Southern Africa and Oceania, influenza virus activity remained low. Some islands in the Pacific reported increased ILI activity. Influenza activity started to increase in South Africa with mainly influenza B viruses detected.
- National Influenza Centres (NICs) and other national influenza laboratories from 90 countries, areas or territories reported data to FluNet for the time period from 02 May 2016 to 15 May 2016 (data as of 2016-05-27 03:46:29 UTC). The WHO GISRS laboratories tested more than 63813 specimens during that time period. 6224 were positive for influenza viruses, of which 2104 (33.8%) were typed as influenza A and 4120 (66.2%) as influenza B. Of the sub-typed influenza A viruses, 938 (79%) were influenza A(H1N1)pdm09 and 249 (21%) were influenza A(H3N2). Of the characterized B viruses, 268 (25%) belonged to the B-Yamagata lineage and 804 (75%) to the B-Victoria lineage.

Respiratory Syncytial Virus

Human RSV infection, the single most important cause of severe respiratory illness in infants and young children and the major cause of infantile bronchiolitis, is the most frequent cause of hospitalization of infants and young children in industrialized countries. In the USA alone, from 85 000 to 144 000 infants with RSV infection are hospitalized annually, resulting in 20%-25% of pneumonia cases and up to 70% of bronchiolitis cases in the hospital. Global RSV disease burden is estimated at 64 million cases and 160 000 deaths every year.

RSV also is a significant problem in the elderly, in persons with cardiopulmonary diseases and in immunocompromised individuals. RSV attack rates in nursing homes in the USA are approximately 5%-10% per year with a 2%-8% case fatality rate, amounting to approximately 10 000 deaths per year among persons >64 years of age. Among elderly persons followed for 3 consecutive winters, RSV

infection accounted for 10.6% of hospitalizations for pneumonia, 11.4% of hospitalizations for obstructive pulmonary disease, 5.4% for congestive heart failure and 7.2% for asthma.

Few population-based estimates of the incidence of RSV disease in developing countries are available, although existing data clearly indicate that the virus accounts for a high proportion of ARIs in children. Studies in Brazil, Colombia and Thailand suggest that RSV causes 20-30% of ARI cases in children from 1-4 years of age, a proportion similar to that in industrialized countries. Another confusing aspect of the epidemiology of RSV infection is the seasonality of the disease. In Europe and North America, RSV disease occurs as well-defined seasonal outbreaks during the winter and spring months. Studies in developing countries with temperate climates, such as Argentina, have shown a similar seasonal pattern. On the other hand, studies in tropical countries often have reported an increase in RSV in the rainy season but this has not been a constant finding.

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10 INTERFERENCES AND INHIBITORS OF PCR

See Interferences and Inhibitors of PCR report.

11 STABILITY ASSAY

The aim of the stability assays is to demonstrate that the characteristics and performance of the **VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection kit** are not altered with the time under normal use due to the influence of different environmental factors such as temperature, transport, sunlight, humidity and vibration.

At first, in order to do that, **an evaluation of VIASURE devices stability in real time and accelerated ageing** has been performed in **VIASURE open platform products to determine the shelf-life and expiry date**. As well as, the remaining stability assays (e.g. stability in extreme conditions, in-use stability and the shipping conditions) were performed on some representative open platform devices, since the obtained data can be extrapolated to all VIASURE Real Time PCR Detection products. See the protocol and acceptance criteria are described in **VIASURE Stability Protocol for Open Platform devices** and **VIASURE Global Stability Report.**

The most critical component in order to estimate the shelf-life and expiry date of VIASURE devices is the **stabilized PCR reaction mix**. Depending on the specifications of the Real Time PCR Platform used, the reaction mix can be placed on different tubes or wells and VIASURE Real Time PCR Detection kit can be marketed on different formats (**See VS-Description of Product Components and Labelling and Annex**):

a) *White 8-well strip or 96-well plate in high or low profile (open platform devices);* which contains in each well all reagents for a 20-µL assay in a lyophilized format by freezing drying (compatibility with the most common Real Time PCR platform (equipment's stated in the Instruction for Use, Annex I).
b) *Transparent 4-well strips*; compatible with Rotor Gene Q Real Time PCR platform (QIAGEN).
c) *Reaction-Mix tube* (which contain 24 reactions, once the Reaction-Mix tube has been re-suspended, the reaction-mix can be added to different wells or tubes of different Real Time PCR platforms, as well).

Due to last two VIASURE devices (b and c; 4-well strips and Reaction-Mix Tubes), contains in each tube the same reagent proportions (TAQ DNA polymerase, target-specific primers and probes, and stabilizing compounds), than VIASURE products for open platform (a, wells in low or high profile which was the first format marketed); we consider that it could be only necessary to perform some additional trials in order to ensure the shelf-life and expiry date and the in-use stability. See the protocol and acceptance criteria described in **VIASURE Stability Protocol for Rotor Gene Q and Reaction-Mix tube.**

In summary, the results from the test that have been carried out showed a long-term stability of kits under the different temperature conditions and the robustness of tests. No influence related to specific targets,

the input sample (DNA or RNA) or differences between monoplex and multiplex assays were observed in the stability assays.

After considering the results, we were able to established 24 months as Product Shelf-life for DNA/RNA kits at temperature range from +2ºC to +40°C since manufacturing date for the device marketed on 8-well strip or 96-well plate in high or low profile (open platform devices), 4-well strips compatible with Rotor Gene Q Real Time PCR platform (QIAGEN) and Reaction-Mix tube.

12 VALIDATION PROCEDURE

Besides that, VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit was evaluated at three independent laboratories with three different Real Time PCR instruments: Bio-Rad CFX96™ Real-Time PCR Detection System, AriaMx Realtime PCR System (Agilent Technologies) and Applied Biosystems 7500 Fast Real-Time PCR System.

The panel samples and controls tested on the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit were previously described in section 3. The arithmetic mean ($\bar{x}$), the standard deviation (σ) and the coefficient of variation (CV%) were calculated and are shown in Tables 55, 56 and 57.

Sample	Pathogen	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive 1	SARS-CoV-2	FAM	29.35	0.44	1.50
Positive 2	Influenza Virus	ROX	29.44	0.14	0.49
Positive 3	RSV Virus	Cy5	32.08	0.02	0.07
Three viruses Positive	SARS-CoV-2	FAM	30.45	0.13	0.42
	Influenza Virus	ROX	31.54	0.26	0.84
	RSV Virus	Cy5	34.40	1.23	3.57
Negative sample	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	25.03	0.12	0.48
Positive Control	SARS-CoV-2	FAM	22.88	0.11	0.49
	Influenza Virus	ROX	26.32	0.05	0.19
	RSV Virus	Cy5	25.29	0.09	0.34
Negative Control	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 55. VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch nº: CFR1XL-EXP.469C, expiry date 2022-07) assay run on the **Bio-Rad CFX96 Touch™ Real-Time PCR Detection System**. (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

Sample	Pathogen	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive 1	SARS-CoV-2	FAM	29.64	0.63	2.14
Positive 2	Influenza Virus	ROX	29.71	0.93	3.13
Positive 3	RSV Virus	Cy5	30.59	0.30	0.99
Three viruses Positive	SARS-CoV-2	FAM	30.27	0.22	0.71
	Influenza Virus	ROX	30.75	0.19	0.63
	RSV Virus	Cy5	31.65	0.21	0.65
Negative sample	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	23.78	0.10	0.44
Positive Control	SARS-CoV-2	FAM	21.93	0.07	0.32
	Influenza Virus	ROX	25.17	0.09	0.37
	RSV Virus	Cy5	23.28	0.10	0.41
Negative Control	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 56. VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07) assay run on the **AriaMx Realtime PCR System (Agilent Technologies).** (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

Sample	Pathogen	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive 1	SARS-CoV-2	FAM	31.22	0.50	1.61
Positive 2	Influenza Virus	ROX	31.45	0.93	2.96
Positive 3	RSV Virus	Cy5	33.40	0.28	0.83
Three viruses Positive	SARS-CoV-2	FAM	31.31	0.49	1.55
	Influenza Virus	ROX	32.23	0.07	0.21
	RSV Virus	Cy5	35.59	0.30	0.84
Negative sample	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	24.73	0.09	0.36
Positive Control	SARS-CoV-2	FAM	22.88	0.32	1.39
	Influenza Virus	ROX	26.72	0.34	1.28
	RSV Virus	Cy5	26.24	0.30	1.14
Negative Control	SARS-CoV-2 Influenza Virus RSV Virus	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 57. VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Batch n°: CFR1XL-EXP.469C, expiry date 2022-07) assay run on the **Applied Biosystems 7500 Fast Real-Time PCR System.** (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg= negative, n.a.= not applicable.

The results have been recorded internally on Excel data sheet "2 Precision CFR1".

In addition, several external clinical evaluation studies will be performed to estimate the clinical sensitivity and specificity. All the assays will be carried out following the instructions described in the test procedure section of the Handbook. The reports and practical feedback provided by final customers will allow us to identify and resolve potential test procedure understanding difficulties.

Besides, we are registered in external quality assessment programmes (EQA) to evaluate VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit. Prior to the submission of the results for EQA programmes, a technical questionnaire which includes questions regarding the laboratory set-up (Extraction and Real Time PCR amplification platforms), assay method and test procedures had been completed.

In conclusion, we consider that the VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit is suitable for its intended use and performs accordingly to the pre-determined criteria.

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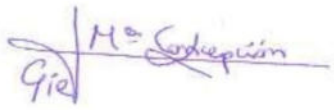
Signature	
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