VIASURE

Real Time PCR Detection Kit

SARS-CoV-2 del 69/70, ORF1ab & N genes

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 del 69/70, ORF1ab & N genes* 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, metrological traceability, clinical evaluation studies, interferences and inhibitors of PCR and stability assays in order to demonstrate that VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit is suitable for its intended use and meet according to pre-determined criteria described in each applicable standard operating procedure (POC):

- POC-67 MDx Project development.
- POC-51 Evaluación de Funcionamiento (Performance Evaluation).
- POC-43 Validation procedure for qPCR products.
- POC-71 Clinical Performance Evaluation MDX.

After the Analytical and Clinical Performance Evaluations, a summary of the results obtained is included in this document.

Any relevant update of new Analytical assays, Clinical evaluation of the product, Scientific References/Literature, participation in External Quality Assurance Services (EQAS) programs (Inter-comparison Studies), or additional evidence obtained after CE/IVD mark approval, will be also updated in this report.

2 PRODUCT SPECIFICATIONS

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).

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 del 69/70, ORF1ab & N genes* 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.

3 ABBREVIATIONS AND DEFINITIONS

Term	**Definition**
Analytical performance study	A study carried out to assess the ability of the *in vitro* diagnostic medical device (IVD) to detect or measure a particular analyte.
Clinical performance study	A study carried out to demonstrate "the ability of the IVD medical device to yield results that are correlated with a particular condition/physiological state in accordance with (the) target population and intended user".
Accuracy	Measurement accuracy covers trueness and precision. Accuracy is affected by a combination of systematic and random effects that contribute as individual components of the total error of measurement.
Trueness	Measurement trueness is affected by systematic error, is normally expressed in terms of bias.
Precision	Measurement precision is affected by random error, is naturally expressed in terms of standard deviation.
Repeatability	"Repeatability" refers to a precision estimate obtained from replicate measurements made in one laboratory by a single analyst using the same equipment and obtained in a single batch of analysis over a short time scale. It gives an indication of the short-term variation in measurement results. Precision under repeatability conditions, often called simply 'repeatability', is also referred to as within-batch or intra-assay precision.
Reproducibility or Intermediate precision	"Reproducibility" or "Intermediate precision" (within-laboratory reproducibility or precision) refers to a precision estimate obtained from replicate measurements made in a single laboratory under different variable conditions: days, runs, lots, operators (intended users) and instruments (different sets of equipment within the same laboratory).
Analytical Sensitivity	Analytical sensitivity refers to the minimum number of copies in a sample that can be measured accurately with an assay.
Linearity	The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
LoD	Limit of Detection, which is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact

Term	Definition
	value. It can be defined as well as the concentration that can be detected with reasonable certainty with a given analytical procedure.
LoB	Limit of Blank is the highest value we expect to see in a series of results on a sample that contains no analyte.
Analytical Specificity	Refers to the ability of an assay to measure on particular organism or substance, rather than others, in a sample.
Metrological traceability	Property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.
Control material	Substance, material or article intended by its manufacturer to be used to verify the performance characteristics of an IVD.
Reference Material (RM)	Material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.
Clinical Sensitivity	The sensitivity of the new test is estimated as the proportion of subjects with the target condition in whom the test is positive.
Clinical Specificity	The specificity of the test is estimated as the proportion of subjects without the target condition in whom the test is negative.
NPV	Negative Predictive Value is the proportion of test negative patients who do not have the target condition.
PPV	Positive Predictive Value is the proportion of test positive patients who have the target condition.
OPA	Overall percentage agreement. The overall percentage agreement (OPA) is defined as the percentage of total subjects where the new test and the non-reference standard agree.
PPA	Positive percentage agreement. PPA is the proportion of non-reference standard positive subjects in whom the new test is positive.
NPA	Negative percentage agreement. NPA is the proportion of non-reference standard negative subjects in whom the new test is negative.
True positive (TP)	A positive test result, that accurately reflects the tested-for activity of an analyte.
False positive (FP)	A test result that erroneously assigns a patient to a specific diagnostic or reference group.

Term	Definition
False negative (FN)	A test result that erroneously excludes someone from a specific diagnostic or reference group.
True negative (TN)	A negative test result, that accurately reflects the tested-for activity of an analyte.
Kappa value	Cohen's kappa coefficient (κ) is a statistic that is used to measure inter-rater reliability (and also intra-rater reliability) when the outcome of interest is measured on a nominal scale.

Table 1. Abbreviations and definitions.

4 SAMPLE COLLECTION, STORAGE AND TRANSPORT

The VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit has been validated on nasopharyngeal swabs collected with synthetic fiber swabs with plastic and placed immediately into a sterile transport tube containing Universal transport medium (UTM). Other types of samples must be validated by the user.

The respiratory specimens must be collected, transport and storage according to appropriate laboratory guidelines. For details, refer to the CDC guidelines (Specimen collection guidelines. Website https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf and Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Website https:// www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html) and the IDSA guideline (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94)

The respiratory specimens should be collected as soon as possible, during the acute phase of illness or at any time during the clinical course (depending on the pathogen), and before antimicrobic therapy begin, if possible. Specimens should be obtained by avoiding touching adjacent tissues and organ secretions that are not of interest and should be collected in a sterile container with or without transport media (depending on sample type). All specimens must be labeled with the name and identification number of the person from whom the specimen was collected, the source of the specimen, and the date and time it was collected. After collection, specimens should be placed in a biohazard bag and transported to the laboratory as soon as possible. The specimens should be transported at 2 to 8°C for up to 72 hours, following the local and national regulations for the transport of pathogen material. For long term transport (more than 72 hours), we recommend shipping at -20°C or lower.

Optimally, all specimen containers should be opened in a biological safety cabinet. Appropriate barriers should be used to prevent exposure of skin and mucous membranes to the specimen. Gloves and a lab coat must always be worn when handling patient specimens. Masks, goggles (or working behind a plastic

shield), and impermeable gowns or aprons must be worn when there is a risk for splashes or droplet formation. Wash your hands once the proceeding has finished.

Specimens submitted for molecular testing must be stored in controlled conditions so that nucleic acids do not degrade during storage. It is recommended to use fresh specimens for the test. The samples can be stored at 2 to 8°C for up to 72 hours or frozen at -20°C or ideally at -70°C for conservation.

Avoid freezing and thawing cycles. Some pathogens from specimens that are frozen and then thawed could be degraded and may result in false-negative test results.

For more information, see **VIASURE Sample collection, transport, storage and stability Report**.

5 REAGENTS AND EQUIPMENT

5.1 NUCLEIC ACID EXTRACTION KITS AND PLATFORMS

Any manual or automatic optimized system from human clinical specimens can be used for RNA from SARS-CoV-2 extraction, following the manufacturer's instructions or the in-house protocol, but must be validated by the user.

The following extraction kits have been validated for RNA extracted from nasopharyngeal swabs:

- MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the KingFisher Flex System instrument (ThermoFisher).
- MagDEA Dx SV kit, using the magLEAD® 12gC instrument (Precision System Science Co).

5.2 REAL TIME PCR PLATFORMS

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit has been validated on the following PCR equipments:

- Applied Biosystems 7500 Fast Real-Time PCR System.
- Bio-Rad CFX96™ Real-Time PCR Detection System
- Agilent Technologies AriaMx Real-Time PCR System.
- DNA-Technology DTprime Real-time Detection Thermal Cycler.
- DNA-Technology DTlite Real-Time PCR System.
- Roche Molecular Diagnostics Cobas z480 Analyzer.
- NEOS-96 qPCR (Linear Chemicals)

6 SCIENTIFIC VALIDITY DETERMINATION

The diagnosis of infectious diseases in humans can be established, mainly, either by direct methods such as microscopy after adequate staining, or by indirect methods such as immunological antigen detection, such as immunochromatography, conventional EIA ("Enzyme Immunoassay"), ELISA ("Enzyme-Linked Immuno Sorbent Assay") or the agglutination test. However, the standard reference technique on which the rest of the diagnostic tests have been based, as it is the most specific, consists in identifying the infection by isolating the pathogen in culture. However, all these techniques have important shortcomings that must be considered.

As an alternative, the trend in diagnostics is to employ newer and more sensitive molecular identification technologies. Molecular diagnostic techniques combine laboratory diagnosis with molecular biology, which has revolutionized the way Public Health Systems investigate human, viral, and microbial genes. Besides, the use of molecular diagnostic techniques it is also serving to deepen the knowledge of the disease and the epidemiological behavior of the pathogens that cause it, which allows taking the appropriate control measures for the patient as well as promoting the rational use of antivirals and antibiotics.

In some laboratories and hospitals, nucleic acid amplification methods have already been established for microbiological diagnosis, generally conventional PCR (also called end-point PCR).

Quantitative real-time PCR (qPCR) allows a faster, more specific, sensitive, reliable and reproducible diagnosis to be achieved from a wide variety of biological samples even with poor quality. The main advantages of qPCR compared to standard PCR are the reduction in assay time, less risk of contamination since it does not require post-PCR analysis, the improvement in sensitivity, as well as the possibility of testing several pathogens at the same time.

The real-time PCR detection products available on the market have progressively evolved towards greater simplicity, leading to increasingly compact and simple formats that minimize the necessary pipetting steps. In this growing trend towards simplicity, there is a wide variety of commercially available kits that include specific probes and primers for the detection of a single pathogen or multiple targets simultaneously in a single tube, which would be called multiplex PCR.

7 ANALYTICAL SENSITIVITY AND LINEARITY

7.1 LINEARITY OF THE ASSAY AND TENTATIVE LIMIT OF DETECTION OR LOD

The 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. Every tenfold dilution was tested in triplicate as well as the

last dilution around the detection limit which was tested 20 replicates. The arithmetic mean ($\bar{x}$), the standard deviation (σ) and the coefficient of variation (CV%) were calculated and detailed in Table 2, 3 and 4.

Example of the amplification plot resulting from an assay run on the Bio-Rad CFX96™ Real-Time PCR Detection System is included in the Instructions for use.

	copies/rxn							
Synthetic HV 69/70 deletion cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**10^1**	**0**
$\bar{x}$ (Ct)	14.61	18.36	22.01	25.54	28.22	32.39	36.19	Neg
σ	0.18	0.25	0.25	0.21	0.12	0.34	0.41	n.a.
CV%	1.26	1.35	1.15	0.83	0.41	1.06	1.13	n.a.

Table 2. Analytical sensitivity was evaluated with synthetic HV 69/70 deletion cDNA and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -FAM channel- (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02), **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of ≥10 cDNA copies per reaction for HV 69/70 deletion. cDNA molecules can be detected with a concentration of ≥10 copies/rxn (positive rate of ≥95%). The lowest concentration of cDNA that yielded positive test results was the tentative considered the LoD.

The qPCR efficiency was estimated at >91.8% (Slope -3.537). Linear regression showed R^2 value of 0.997.

	copies/rxn							
Synthetic *ORF1ab* gene cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**10^1**	**0**
$\bar{x}$ (Ct)	14.85	18.08	21.48	24.75	28.38	31.63	35.05	Neg
σ	0.24	0.08	0.05	0.27	0.23	0.16	0.11	n.a.
CV%	1.58	0.42	0.25	1.07	0.79	0.49	0.30	n.a.

Table 3. Analytical sensitivity was evaluated with synthetic ORF1ab gene cDNA and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -ROX channel- (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02), **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of ≥10 cDNA copies per reaction for *ORF1ab* gene. cDNA molecules can be detected with a concentration of ≥10 copies/rxn (positive rate of ≥95%). The lowest concentration of cDNA that yielded positive test results was considered the tentative LoD.

The qPCR efficiency was estimated at >97.7% (Slope -3.378). Linear regression showed R^2 value of 1.000.

	copies/rxn							
Synthetic *N* gene cDNA	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**10^1**	**0**
$\bar{x}$ (Ct)	16.02	19.41	22.79	26.04	29.96	33.38	37.76	Neg
σ	0.09	0.06	0.22	0.06	0.18	0.66	1.58	n.a.
CV%	0.54	0.29	0.95	0.23	0.61	1.98	4.18	n.a.

Table 4. Analytical sensitivity was evaluated with synthetic *N* gene cDNA and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -Cy5 channel- (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02), **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of ≥10 cDNA copies per reaction for N2 region. cDNA molecules can be detected with a concentration of ≥10 copies/rxn (positive rate of ≥95%). The lowest concentration of cDNA that yielded positive test results was the tentative considered the LoD.

The qPCR efficiency was estimated at >90.1% (Slope -3.583). Linear regression showed R^2 value of 0.993.

The analytical sensitivity (tentative LOD or linearity) of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was also tested on Cobas z480 Analyzer (Roche Molecular Diagnostics), DTprime Real-time Detection Thermal Cycler (DNA-Technology), DTLite Real-time Detection Thermal Cycler (DNA-Technology), and Applied Biosystems 7500 Fast Real-Time PCR System. The results for all targets match with the LoD which a positive rate of 95% (≥10 copies/rxn for all targets).

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

In addition, the analytical sensitivity of the endogenous IC (human *RNase P* gene) was determined by testing a series of ten-fold dilutions containing a known concentration (ranging from 10^7 to 10^2 copies per reaction) of the plasmid pLenti-RNP Positive Control (GenBank accession number U77665.1) that contains a portion of the *RNasa P* gene (a single copy gene present in the human genome). Every tenfold dilution was tested in triplicate as well as the last dilution around the detection limit which was tested 20 replicates. The arithmetic mean ($\bar{x}$), the standard deviation (σ) and the coefficient of variation (CV%) were calculated and detailed in Table 5.

	copies/rxn						
pLenti-RNP Positive Control	**10^7**	**10^6**	**10^5**	**10^4**	**10^3**	**10^2**	**0**
$\bar{x}$ (Ct)	21.58	24.43	28.01	31.17	34.81	36.47	Neg
σ	0.12	0.36	0.02	0.07	0.51	0.02	n.a.
CV%	0.54	1.46	0.08	0.22	1.47	0.06	n.a.

Table 5. Analytical sensitivity was evaluated with the plasmid pLenti-RNP Positive Control and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -HEX channel- (Batch nº: SUK2XL-EXP.517B, expiry date 2023-02), **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of ≥1000 DNA copies per reaction for *RNasa P* gene. DNA molecules can be detected with a concentration of ≥1000 copies/rxn (positive rate of ≥95%) and 100 copies/rxn (positive rate of ≥66%).

The qPCR efficiency was estimated at >107.1% (Slope -3.150). Linear regression showed R^2 value of 0.991.

The analytical sensibility of the endogenous IC (human *RNase P* gene) with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was also tested on Cobas z480 Analyzer (Roche Molecular Diagnostics), DTprime Real-time Detection Thermal Cycler (DNA-Technology), DTLite Real-time Detection Thermal Cycler (DNA-Technology), and Applied Biosystems 7500 Fast Real-Time PCR System. The results for all targets match with the LoD which a positive rate of 95% (≥1000 copies/rxn for *RNasa P* gene).

The results have been recorded on Excel data sheet "1 Curves-Analytical Sensitivity - SUK2"

7.2 LIMIT OF DETECTION (LOD)

Different analytical sensitivity assays were performed to establish and confirm the LoD of the kit with several workflows, considering the entire procedure (sample preparation, RNA extraction method and Real Time PCR Instrument).

7.2.1 Workflow 1: MagMAX™ Viral/Pathogen II (MVP II) + Bio-Rad CFX96™

7.2.1.1 Nasopharyngeal swabs

2019 Novel Coronavirus, Strain:2019-nCoV/USA-WA-1/2020 (ATCC-VR-1986HK)

Besides, another analysis was performed to determine the LoD of SARS-CoV-2 (*ORF1ab* and *N* genes targets) in genome copies per reaction (genome copies/rxn). The LoD was determined by testing five times four negative clinical nasopharyngeal 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 to 0.5 genome copies/µL). The four spiked samples were extracted with MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Batch n°01032021), using the KingFisher Flex System instrument (ThermoFisher) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02) 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 and 7.

	Genome copies / rxn (VTM, Vircell)	
Negative samples spiked with quantified heat-inactivated culture 2019 Novel Coronavirus (ATCC-VR-1986HK) – *ORF1ab* gene	**40**	**20**
$\overline{x}$ (Ct)	36.15	37.70
σ	1.53	1.76
CV%	4.22	4.66
n	20/20	11/20

Table 6. The LoD was determined with quantified heat-inactivated culture 2019 Novel Coronavirus and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -ROX channel- ORF1ab gene (Batch n°: Batch n°: SUK2XL-EXP.517B, expiry date 2023-02). **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of **40 genome copies per reaction for SARS-CoV-2 (*ORF1ab* gene)** with a positive rate of ≥95%.

	Genome copies / rxn (VTM, Vircell)	
Negative samples spiked with quantified heat-inactivated culture 2019 Novel Coronavirus (ATCC-VR-1986HK) – *N* gene	**40**	**20**
$\overline{x}$ (Ct)	36.79	38.40
σ	1.42	0.98
CV%	3.87	2.55
n	19/20	11/20

Table 7. The LoD was determined with quantified heat-inactivated culture 2019 Novel Coronavirus and *VIASURE SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -Cy5 channel- N gene (Batch n°: Batch n°: SUK2XL-EXP.517B, expiry date 2023-02). **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of **40 genome copies per reaction SARS-CoV-2 (*N* gene)** with a positive rate of ≥95%.

Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528)

To determine the LoD of SARS-CoV-2 (*ORF1ab* and *N* genes *and* HV 69/70 deletion targets) in genome copies per reaction (genome copies/rxn). The LoD was determined by testing five times four negative clinical pharyngeal (throat) swabs (viral transport medium, VTM- Vircell-) (total twenty times) spiked with a

known concentration of Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528) (103907) (which were at the detection limit). The dilutions contained a known concentration of synthetic control (2.0 genome copies/µL to 0.5 genome copies/µL). The four spiked samples were extracted with MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Batch n°01032021), using the KingFisher Flex System instrument (ThermoFisher) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02) 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 Tables 8, 9 and 10.

	Genome copies/ rxn (VTM, Vircell)	
Negative samples spiked with Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528) (103907) – *ORF1ab* gene	**40**	**20**
$\overline{x}$ (Ct)	36.04	36.71
σ	1.31	1.48
CV%	3.63	4.04
n	19/20	16/20

Table 8. The LoD was determined with quantified heat-inactivated Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528) and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -ROX channel- ORF1ab gene (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02). **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of **40 genome copies per reaction for SARS-CoV-2 (*ORF1ab* gene)** with a positive rate of ≥95%. **This concentration was finally considered the LoD for SARS-CoV-2 (*ORF1ab* gene).**

	Genome copies/ rxn (VTM, Vircell)	
Negative samples spiked with Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528) (103907) – *N* gene	**80**	**40**
$\overline{x}$ (Ct)	37.60	37.21
σ	1.41	1.66
CV%	3.76	4.47
n	20/20	13/20

Table 9. The LoD was determined with quantified heat-inactivated Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528) and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -Cy5 channel- N gene (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02). **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of **80 genome copies per reaction SARS-CoV-2 (*N* gene)** with a positive rate of ≥95%. **This concentration was finally considered the LoD for SARS-CoV-2 (*N* gene).**

	Genome copies/ rxn (VTM, Vircell)	
Negative samples spiked with Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528) (103907)– HV 69/70 deletion	**40**	**20**
$\overline{x}$ (Ct)	37.16	37.07
σ	1.66	1.15
CV%	4.47	3.11
n	19/20	18/20

Table 10. The LoD was determined with quantified heat-inactivated Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528) and VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit -FAM channel- HV 69/70 deletion (Batch nº: SUK2XL-EXP.517B, expiry date 2023-02). **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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit showed a detection limit of **40 genome copies per reaction for HV 69/70 deletion** with a positive rate of ≥95%. **This concentration was finally considered the LoD for HV 69/70 deletion.**

The results have been recorded on Excel data sheet "1 Curves-Analytical Sensitivity SUK2".

8 NO TEMPLATE CONTROL ASSAY

To determine the "limit of blank" (LoB), non-template control assay was performed. For this assay, at least 96 reaction mixes were reconstituted with rehydration buffer. Afterwards the RNAse/DNAse Water free was added and reactions were run on Cobas z480 Analyzer (Roche Molecular Diagnostics), DTprime Real-time Detection Thermal Cycler (DNA-Technology), AriaMx Real-Time PCR System (Agilent Technologies), and NEOS-96 qPCR (Linear Chemicals). In particular, four 96-well plates of VIASURE Real Time PCR Detection device without endogenous IC (Batch nº: SUK2XL-EXP.517B, expiry date 2023-02).

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.

The results have been internally recorded on Excel data sheet "1 Curves-Analytical Sensitivity SUK2 (Negative sheet)".

9 PRECISION

To determine the precision, intra-assay (repeatability), inter-assay (reproducibility) and inter-batches assay were performed with nasopharyngeal swabs collected in UTM 2 ml. For all the assays, one positive sample

for SARS-CoV-2 (non HV 69/70 deletion), one positive sample for SARS-CoV-2 (HV 69/70 deletion), and two additional positive and negative samples for all targets (SARS-CoV-2 (non HV 69/70 deletion), and SARS-CoV-2 (HV 69/70 deletion)) were tested (Positive samples with different Ct values above LOD, samples with a Ct value ≤ 35).

The panel of samples were extracted with MagDEA Dx SV kit, using the magLEAD® 12gC instrument (Precision System Science Co.) (Batch nº 23M020, Expiry date 2022-11) following manufacturer's instructions and were analyzed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº: SUK2XL- EXP.517B, expiry date 2023-02).

Spiked specimens were stored frozen at -20 or -80ºC and were totally thawed, brought to room temperature and homogenised before testing. RNA/DNA samples were stored at -20 or -80ºC 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 were showed in Tables 11, 12 and 13.

9.1 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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit and Bio-Rad CFX96™ Real-Time PCR Detection System. 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 the Negative Control. Table 11 shows the results obtained in this assay.

Sample	Target	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	34.13	0.65	1.89
	SARS-CoV-2 (*ORF1ab* gene)	ROX	30.38	0.15	0.48
Positive 2 Three targets positive	SARS-CoV-2 (*N* gene)	Cy5	32.86	0.45	1.36
	SARS-CoV-2 (HV 69/70 deletion)	FAM	35.16	0.88	2.49
Pool Positive Negative sample	SARS-CoV-2 (*ORF1ab* gene)	ROX	32.10	0.39	1.20
	SARS-CoV-2 (*N* gene)	Cy5	34.61	1.53	4.42
	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
Negative sample Positive Control	Endogenous Internal Control	HEX	23.75	0.23	0.96
	SARS-CoV-2 (HV 69/70 deletion)	FAM	24.20	0.11	0.44
Positive Control Negative Control	SARS-CoV-2 (*ORF1ab* gene)	ROX	25.19	0.15	0.61
	SARS-CoV-2 (*N* gene)	Cy5	22.19	0.18	0.80
	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
Negative Control Positive	Endogenous Internal Control	HEX	Neg	n.a.	n.a.
	SARS-CoV-2 (HV 69/70 deletion)	FAM	34.13	0.65	1.89

Table 11. Intra-assay reproducibility of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02). (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a.= not applicable.

9.2 INTER-ASSAY

The inter-assay values were determined by testing the different samples on three different days by three different operators with the VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit run on Bio-Rad CFX96™ Real-Time PCR Detection System. In a similar way, the Positive and Negative controls (PC and NC, respectively) were also analyzed (Table 12).

Sample	Target	Viasure channel	$\bar{x}$ (Ct)	Σ	CV%
Positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	33.94	0.62	1.83
	SARS-CoV-2 (*ORF1ab* gene)	ROX	30.55	0.32	1.04
	SARS-CoV-2 (*N* gene)	Cy5	32.64	0.33	1.02
Three targets positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	34.32	0.55	1.62
	SARS-CoV-2 (*ORF1ab* gene)	ROX	31.71	0.38	1.21
	SARS-CoV-2 (*N* gene)	Cy5	33.43	0.84	2.51
Negative sample	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	23.91	0.27	1.14
Positive Control	SARS-CoV-2 (HV 69/70 deletion)	FAM	24.10	0.12	0.50
	SARS-CoV-2 (*ORF1ab* gene)	ROX	25.09	0.11	0.45
	SARS-CoV-2 (*N* gene)	Cy5	22.16	0.13	0.58
Negative Control	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 12. Inter-assay reproducibility of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02). (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg= negative, n.a.= not applicable

9.3 INTER-BATCH (INTERMEDIATE PRECISION)

The inter-batch values were determined with 3 replicates at three concentrations of the different samples by using three batches of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batches n°: SUK2XL-EXP.517B, SUK2XL-EXP.517C and SUK2XL-EXP.517D expiry dates 2023-02, 2023-03 and 2023-03) run on AriaMx Realtime PCR System (Agilent Technologies). The results are described below in Table 13. In addition, the Positive Control (PC) and Negative Control (NC) were also analysed in a similar way.

Sample	Target	Viasure channel	$\bar{x}$ (Ct)	Σ	CV%
Positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	33.78	1.17	3.47
	SARS-CoV-2 (*ORF1ab* gene)	ROX	29.75	0.59	2.00
	SARS-CoV-2 (*N* gene)	Cy5	30.59	0.64	2.11
Three targets positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	33.82	1.26	3.72
	SARS-CoV-2 (*ORF1ab* gene)	ROX	31.78	1.00	3.13
	SARS-CoV-2 (*N* gene)	Cy5	31.13	0.92	2.96
Negative sample	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	22.75	0.40	1.74
Positive Control	SARS-CoV-2 (HV 69/70 deletion)	FAM	23.26	0.37	1.61
	SARS-CoV-2 (*ORF1ab* gene)	ROX	24.00	0.34	1.43
	SARS-CoV-2 (*N* gene)	Cy5	20.21	0.37	1.85
Negative Control	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 13. Inter-batch reproducibility of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batches n°: SUK2XL-EXP.517B, SUK2XL-EXP.517C and SUK2XL-EXP.517D expiry dates 2023-02, 2023-03 and 2023-03). (**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 SUK2".

10 ANALYTICAL SPECIFICITY AND REACTIVITY

The specificity of each assay was assessed by using publicly available nucleotide sequence databases and search and/or alignment tools as BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), Maftt Aligment (http://mafft.cbrc.jp/alignment/server/) and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast)). The bioinformatic analyses showed that mostly the targeted pathogens SARS-CoV-2. Besides, they did not match with other nucleic acid sequences belonging to human or microbial species (Human coronaviruses (HCoVs) strains: 229E, HKU1, NL63, OC43, SARS-CoV and MERS-CoV).

Besides, *in silico* analysis for VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit is ongoing.

10.1 ANALYTICAL SPECIFICITY AND MICROBIAL INTERFERENCE

Analytical Specificity wet testing

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 of the VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02) with genomic RNA/DNA of the selected pathogens was observed (Table 14). These assays were run on Bio-Rad CFX96™ Real-Time PCR Detection System.

Cross-reactivity testing (wet testing)					
Human Adenovirus types 1-5, 8, 15, 31, 40 and 41	-	Influenza A/New Caledonia/20/99(H1N1) virus	-	*Legionella longbeachae*	-
Bocavirus	-	Influenza A/California/7/2009(H1N1)pdm09	-	*Legionella micdadei*	-
Bordetella bronchiseptica	-	Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-	*Legionella pneumophila*	-
Bordetella holmesii	-	Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-	Human metapneumovirus A and B	-
Bordetella parapertussis	-	Influenza A/Victoria/210/2009 (H3N2)	-	*Moraxella catarrhalis*	-
Bordetella pertussis	-	Influenza A/Thüringen/5/17 (H3N2) virus	-	*Mycoplasma pneumoniae*	-
Chlamydia caviae	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-	*Mycobacterium tuberculosis* not rifampin resistant	-
Chlamydia psittaci genotype A and C	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-	Human parainfluenza 1, 2, 3 and 4 viruses	-
Chlamydophila pneumoniae CM-1	-	Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus	-	*Pneumocytis jirovecii* Type A1 and g885652	-
Human coronavirus 229E, OC43, NL63 and HKU1	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus	-	Human rhinovirus type C	-
MERS Coronavirus	-	Influenza A/Anhui/1/2013 (H7N9) virus	-	*Staphylococcus aureus* subsp. *aureus*	-
SARS Coronavirus Strain Frankfurt 1	-	Influenza B/Brisbane/60/2008	-	*Staphylococcus epidermidis*	-
Enterovirus 68 and 71	-	Influenza B/Florida/04/06 virus	-	*Streptococcus pneumoniae* Z022	-
Enterovirus Echovirus 11 and 30	-	Influenza B/Phuket/3073/2013 virus	-	*Streptococcus pyogenes*	-
Enterovirus Coxsackievirus A24, A9 and B3	-	*Legionella bozemanii*	-	*Streptococcus salivarius*	-
Haemophilus influenzae MinnA	-	*Legionella dumoffii*	-	Respiratory syncytial virus (RSV) A and B	-

Table 14. Reference pathogenic microorganisms used in this study performed at CerTest facility.

The results have been internally recorded on Excel "3 Cross-reactivity testing Reactivity and Specificity SUK2".

Anlytical specificity *In silico* analysis

In addition to microorganism wet testing, *in silico* analysis was performed to assess the specificity of the assay in relation to the microorganisms listed in Table 15. BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast) analyses over each primer and/or probe against the sequences from NCBI Genbank Nucleotide Database (from NCBI Genbank Nucleotide Database (https://www.ncbi.nlm.nih. gov/genbank/), were performed.

The organism included in this *in silico* cross-reactivity analysis can be grouped depending on the reasoning behind the inclusion:

Other high priority pathogens from the same genetic family	Human coronavirus 229E, Human coronavirus OC43, Human coronavirus HKU1, Human coronavirus NL63, SARS-coronavirus and MERS-coronavirus.
High priority organisms likely in the circulating area	Adenovirus (e.g. C1 Ad. 71), Human Metapneumovirus (hMPV), Parainfluenza virus 1-4, Influenza A, Influenza B, Enterovirus (e.g. EV68), Respiratory syncytial virus, Rhinovirus, Chlamydia pneumoniae, Haemophilus influenzae, Legionella pneumophila, Mycobacterium tuberculosis, Streptococcus pneumonia, Streptococcus pyogenes, Bordetella pertussis, Mycoplasma pneumoniae, Pneumocystis jirovecii (PJP), Candida albicans, Pseudomonas aeruginosa, Staphylococcus epidermidis and Streptococcus salivarius
High priority organisms, including organisms commonly found in the clinical matrix	Influenza C, Parechovirus, Corynebacterium diphtheriae, Legionella non-pneumophila, Bacillus anthracis (Anthrax), Moraxella catarrhalis, Neisseria elongata and meningitidis, Leptospira, Chlamydia psittaci, Coxiella burnetii (Q-Fever) and Staphylococcus aureus.

Table 15. Organisms assessed in silico Cross-Reactivity analysis.

Excepting SARS Coronavirus, all the analysed Organisms and sequences showed:

- Insufficient query cover to consider a high homology between sequences.
- Homologue regions split in several sequence fragments spamming along the organism sequence, making PCR product amplifications unlikely.
- No amplification product resulting in Primer BLAST analysis or too lengthy (>800 nt) to hinder SARS-CoV-2 amplification and detection. Particularly,
    - *Leptospira* sequences CP021412.1 and CP000348.1 (GenBank ID) align with *N* gene forward primer with 5 mismatches and in resulting product length of 3326 nt.
    - *Staphylococcus aureus* sequence LS483317.1 (GenBank ID) aligns with *ORF1ab* gene reverse primer with 4 mismatches and in resulting product length of 828 nt.

Based on the *in silico* analysis, it is predicted that the assay may cross-react with SARS-CoV, SARS-like coronaviruses, and animal coronaviruses (Bat and Pangolin Coronaviruses), however, the aligned sequences show several mismatches. Besides, these animal coronaviruses have either not been identified in humans before or are considered eradicated, dating the last SARS coronavirus official diagnosis back to 2004. Therefore, the homology of primers and probes sequences to these viruses should cause no interference in the SARS-CoV-2 detection.

The *in-silico* analysis performed to check the cross reactivity is detailed on **VIASURE SARS-CoV-2 & UK Variant (S UK, ORF1ab and N genes) in silico 100621 rev.00.**

10.2 ANALYTICAL REACTIVITY

Analytical Reactivity wet testing

The reactivity of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated against RNA from Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1, Human 2019-nCoV strain 2019-nCoV/Italy-INMI1, SARS-CoV-2 strain 2019nCoV/USA-WA1/2020, SARS-CoV-2 BetaCoV/Berlin/ChVir1670/2020_IsolatBER, SARS-CoV-2 BetaCoV/Munich/ChVir984/2020, SARS-CoV-2 BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER and synthetic RNA controls for four variants of the SARS-CoV-2 virus: MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020), MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1), B.1.1.7_710528 (Twist Synthetic SARS-CoV-2 RNA Control 14) and B.1.1.7_601443 (Twist Synthetic SARS-CoV-2 RNA Control 15), as the templates (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02).

The results have been internally recorded on Excel "3 Cross-reactivity testing Reactivity and Specificity SUK2".

Analytical Reactivity *in silico* assay

To validate the diagnostic aptitude of VIASURE SARS-CoV-2 ORF1ab and N targets design, primers and probes were analysed for their specificity with the SARS-CoV-2 sequences available in public Database GISAID, as it provides complete, high quality and up-to-date information and is considered as the reference SARS-CoV-2 nucleotide sequence database. The sequences included in this analysis were published in GISAID public database, with 1,298,419 complete, high coverage (<1% Ns, <0.05% unique amino acid mutations not seen in other sequences in the database, and no insertion/deletion unless verified by submitter) SARS-CoV-2 sequences as of the 10th June 2021. This study also includes a specific analysis of VIASURE SARS-CoV-2 & UK ORF1ab and N2 targets design over SARS-CoV-2 variants (See SARS-CoV-2 variants of concern and variants under investigation in England: technical briefing 15, from Public Health England, and available from SARS-CoV-2 variants of concern and variants under investigation (publishing.service.gov.uk). The sequences used for this particular analysis have been obtained from the classification of the variants carried out by the GISAID database. The sequences have the same characteristics (high coverage) and follow identical analysis as that described for the global analysis.

Additionally, an inclusivity evaluation of primers and probes of the del69-70 target was performed over the SARS-CoV-2 variants that carry the aforementioned deletion, as is the case of B.1.1.7, B.1.1.7 with E484K, B.1.258, B.1.1.298, B.1.525 and C.36.3. The same inclusivity evaluation was performed over the remaining variants that do not carry the deletion (https://www.gov.uk/government/publications/investigation-of-novel-sars-cov-2-variant-variant-of-concern-20201201#history).

GISAID database public sequences (up to the 10th May 2021) were analysed using an in-house software based on Biopython analysis queries.

To sum up: >95% of the analysed GISAID database complete alignments will result in amplification of a PCR product for the detection of SARS-COV2, 96.9% of the analysed GISAID sequences containing del69-70 will

result in a proper PCR product for detection, >99% of the analysed GISAID sequences lacking del69-70 will not result in the proper PCR product. To sum up, >99% of the analysed GISAID database complete alignments will result in amplification of a PCR product.

- 95.93% (1,245,555/1,298,419) of the analysed sequences published in GISAID database can be detected with 100% homology with both ORF1ab and N2 sets of primers and probes.
- Of the remaining 4,07% (54,749/1,298,419) sequences that have not 100% homology, 54,320/54,749 of the analysed sequences have only mutated one of the two target gene sets of primers and probes and therefore can be detected with 100% homology with the other gene.
- 0.033% (429/1,298,419) alignments did not show 100% homology with either of the primers and probe sets. However: these 429 alignments were individually analysed; 123 were determined to correctly generate an amplification product in a PCR setting with at least one of the targets and 329 (0.03%) sequences were determined to not be detectable using either ORF1ab or N targets primers and probes.

This *in-silico* inclusivity analysis is detailed on **VIASURE SARS-CoV-2 & UK Variant (S UK, ORF1ab and N genes) in silico 100621 rev.00**.

To continuously monitor the diagnostic aptitude of VIASURE SARS-CoV-2 & UK ORF1ab and N2 targets designs, GISAID public database is continually being analyzed using our in-house software based on Biopython analysis queries.

A summary of the updated data of the *in silico* analysis will be included in the Post Market Surveillance report (PMS).

11 METROLOGICAL TRACEABILITY

The metrological traceability of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* 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 HV 69/70 deletion, *S* gene: NCO2.4PC, FAM channel,
    - Name of synthetic cDNA fragment for *ORF1ab gene*: NCO4PC, ROX channel
    - Name of synthetic cDNA fragment for *N gene*: NCO9PC, Cy5 channel.

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

2. SARS-CoV-2 and Endogenous Internal Plasmid Controls:
    - 2019-nCoV_N_Positive Control (Ref. 10006625, Integrated DNA Technologies, IDT). This control plasmid contains the complete nucleocapsid gene from 2019-nCoV (Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome (GenBank: NC_045512.2)).
    - pLenti-COR, lentiviral vector which contains gene fragments from the viral RNA genome of SARS-CoV-2 (SARS-CoV-2 isolate Wuhan-Hu-1 (GenBank accession number MN908947.3).
    - Hs_RPP30 Positive Control (Ref. 10006626, Integrated DNA Technologies, IDT). This control plasmid contains a portion of the *RPP30* gene (a single copy gene present in the human genome).
    - pLenti-RNP, lentiviral vector which contains human *RPP30* gene fragment (GenBank accession number U77665.1).

3. Control materials: external controls are used to evaluate the good performance of the test, since they are treated in parallel with test specimen and undergo the entire process. Different materials were added to the reaction to check that the result obtained was the expected. Control materials used:
    - AccuPlex™ SARS-CoV-2, Flu A/B and RSV Reference Material Kit (Ref. 0505-0174, SeraCare Life Sciences Inc.).
    - AccuPlex™ SARS-CoV-2 Verification Panel (Ref. 0505-0129, SeraCare Life Sciences Inc.).
    - Research Reagent for SARS-CoV-2 RNA (Ref. 19/304, NIBSC).
    - 2019 novel coronavirus (SARS-CoV-2) Working Reagent for Nucleic Acid Amplification Testing (NAT) (Ref. 20/110, NIBSC).
    - VIASURE Viral SARS-CoV-2 Positive Control Kit (Certest Biotec S.L.).

4. Reference strains: the validity of obtained results during the product validation has been verified using different reference strains for each pathogen. Strains used:
    - 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 four variants of the SARS-CoV-2 virus: MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020), MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1), B.1.1.7_710528 (SARS-CoV-2 RNA Control 14 UK Variant) and B.1.1.7_601443 (SARS-CoV-2 RNA Control 15 UK Variant) (Twist Bioscience Corporation, Ref 102019, 102024, 103907 and 103909: Twist Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1), 2 (MN908947.3), 14 (B.1.1.7_710528) and 15(B.1.1.7_601443)).
    - 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.

  - Heat inactivated SARS-CoV-2 strain 2019nCoV/USA-WA1/2020 (ATCC® VR1986HK™).
  - ATCC Genomic RNA from Severe acute respiratory syndrome-related coronavirus 2. Strain: 2019-nCoV/USA-WA1/2020 (ATCC® VR-1986D™)
  - Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N (ATCC® VR-3276SD™)

- 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.
- Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020 (BEI Resources, Ref. NR-52285).
- SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated (BEI Resources, NR-52286).
- Quantitative Synthetic RNA from SARS-Related Coronavirus 2: ORF, E, N (BEI Resources, NR-52358).
- SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (BEI Resources, NR-52287).

5. 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).
   - **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 9. External Quality Assessment Programs in this report.

12 VALIDATION OF ASSAY CUT-OFF

The selection of the Ct limit (Ct value over which the sample can be considered positive) for VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit has been based on the analytical study carried out considering all data collected from all the available Real Time PCR Detection products of the VIASURE line.

The results of this study can be found in the document: **VIASURE Analytical Sensitivity Report 0819 rev.00.**

13 CLINICAL SENSITIVITY AND SPECIFICITY

In order to determine the clinical diagnostic accuracy of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit, different Multicenter Evaluation will be conducted through collaboration the National&International Microbiology Departments from different entities.

- **Evaluation of the correct detection of the SARS-CoV-2 B.1.1.7 variant with the VIASURE realtime PCR assays aimed at detecting SARS-CoV-2 RNA in clinical samples.**

This evaluation was carried out with the collaboration from "Servicio de Microbiología" of Fundación Jiménez Díaz, Madrid and carried out retrospectively at the CerTest Biotec facilities.

The clinical performance of VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was tested using 60 respiratory clinical samples (nasopharyngeal swabs). From the total of samples, 20 were SARSCoV-2 negative, 20 were SARS-CoV-2 "no UK variant" and 20 were UK variant. The group of B.1.1.7 variant was confirmed by sequencing while the group classified as non-UK variant was tested with the TaqPath COVID-19 assay without confirmation by sequencing. Three samples from the non-uk variant group were negative for all tested products. It was decided to eliminate these samples to perform the analysis due to doubts about the integrity of the samples.

The RNA of the virus was extracted using 200 µL of the clinical samples with the KingFisher Flex automated system with the MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit, using the KingFisher Flex System instrument (ThermoFisher) following the manufacturer's instructions. Eluates were analysed with the

VIASURE assays with the CFX96™ Real-Time PCR instrument (BioRad) thermocycler and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº: SUK2XL-EXP.517B, expiry date 2023-02).

The 20 negative SARS-CoV-2 clinical samples included in the study and the group of SARSCoV-2 B.1.1.7 variant were correctly detected. From the group of "no-UK" variant, 15 were correctly detected and 2 were detected as positive for UK variant as it was observed amplification in the Suk target. These two incongruent samples were sequenced and the VIASURE result was confirmed indicating that the TaqPath COVID-19 CE-IVD RT-PCR Kit molecular assay is reporting 2 UK variant false negative (Tables 16 to 18).

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit	**Sequencing results**		
	+	**-**	**Total**
+	20	2*	22
-	0	35	35
Total	20	37	57

Table 16. VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit results compared to TaqPath COVID-19 and sequencing for SARS-CoV-2 B1.1.7 variant detection and differentiation.

*These two samples were sequenced, and the result obtained by the VIASURE assay was confirmed indicating that the TaqPath COVID-19 molecular assay reported 2 UK variant false negative for the group of UK variant.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit	**Sequencing results**		
	+	**-**	**Total**
+	15	0	15
-	2*	40	42
Total	17	40	57

Table 17. VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit results compared to TaqPath COVID-19 and sequencing for SARS-CoV-2 nonB1.1.7 variant detection and differentiation.

* These two samples were sequenced, and the VIASURE assay result was confirmed indicating that the TaqPath COVID-19 molecular assay reported 2 UK variant false positive for the group of non-UK variant.

Pathogen	Overall agreement	TP	TN	FP	FN	SE	SP	PPV	NPV
UK variant	>99%	22	38	0	0	1 (0.84-1)	1 (0.90-1)	1 (0.84-1)	1 (0.90-1)
Non-UK variant	>99%	18	42	0	0	1 (0.78-1)	1 (0.91-1)	1 (0.78-1)	1 (0.91-1)

Table 18. True positive and negative values, false positive and negative values, sensitivity, specificity, PPV, NPV values for VIASURE *SARS-CoV-2 UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit compared to reference assay.

In this evaluation it was possible to analyse 57 nasopharyngeal clinical samples previously characterized with a reference molecular assay (TaqPath COVID-19 CE-IVD RT-PCR Kit). In the study it was included 3 groups of samples. A first group characterized as negative for SARS-CoV-2, a second group characterized as SARS-CoV-2 non-UK variant and a third group characterized as SARS-COV-2 UK variant. This last group was also confirmed by sequencing.

Regarding the VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit, VIASURE assays showed an overall agreement of 96.6% with the TaqPath COVID-19 CE-IVD RT-PCR Kit. Incongruent results were obtained in two samples and after sequencing it was verified that VIASURE assay reported the true values.

With this evaluation, it was possible to observe that the VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit proved to be as sensitive and more specific than the reference assay. It has proven to be a good tool for the diagnostic of SARS-CoV-2 and the differentiation of the B.1.1.7 variant among the worldwide circulating SARS-CoV-2 strains.

For more details, please see individual Report: "Evaluation of the correct detection of the SARS-CoV-2 B.1.1.7 variant with the VIASURE Realtime PCR assays aimed at detecting SARS-CoV-2 RNA in clinical samples." named file: **VS-EV SARS-CoV-2 UK variant Madrid 0321.**

- **Evaluation of the correct detection of the presence or absence of the 69-70 deletion in the S gene of SARS-CoV-2 in different lineages with the VIASURE real-time PCR assays aimed at detecting SARS-CoV-2 RNA in clinical samples.**

The objective of this study was to verify the correct detection of the HV 69-70 deletion present in SARS-CoV-2 variants (mainly associate with the B.1.1.7) with the VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit. This evaluation was carried out with the collaboration from "Servicio de Microbiología" of the Hospital Universitario Marqués de Valdecilla, Santander and carried out retrospectively at the CerTest Biotec facilities.

The Microbiology Service routinely receives and analyses clinical respiratory (nasopharyngeal swabs) samples collected in 2 mL of UTM from patients with signs and symptoms of SARS-CoV-2. The clinical diagnosis of these samples is carried out with the VIASURE *SARSCoV-2* Real Time PCR Detection Kit test. Due to the appearance of the British variant and the control that is to be carried out on it (following the instructions of the Ministry of Health), in the case of obtaining a low Ct (<30), the samples are analysed with the TaqPath COVID-19 CE-IVD RT-PCR Kit molecular assay. If no amplification of the *S* gene is obtained, the acid nucleic is sent to be sequenced to confirm as a B.1.1.7 variant.

This study was carried out with 100 clinical samples analysed with the workflow described before. All samples were confirmed by sequencing. From the total of samples, 50 were SARS-CoV-2 "no UK variant" and 50 were UK variant. From the group of no UK variant it was included two samples that were *S* gene

negative with the TaqPath COVID-19 assay therefore, presumptive UK variant but after sequencing confirmed as non-UK variant. The SARS-CoV-2 clusters included in the study were: A, A.2, A.5, B.1, B.1.157, B.1.1.282, B.1.1.7, B.1.223, B.1.1.177.

The RNA of the virus was extracted using 200 µL of the clinical samples with the KingFisher Flex automated system with the MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit, using the KingFisher Flex System instrument (ThermoFisher) following the manufacturer's instructions. Eluates were analysed with the VIASURE assays with the CFX96™ Real-Time PCR instrument (BioRad) thermocycler and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº: SUK2XL-EXP.517B, expiry date 2023-02).

A total of 100 nasopharyngeal clinical samples were used in this evaluation. Following initial diagnosis, 50 were SARS-CoV-2 "no UK variant" and 50 were B.1.1.7. From the group of no UK variant it was included two samples that were *S* gene negative with the TaqPath COVID-19, therefore, possible B.1.1.7 but after sequencing confirmed as non-UK variant. Following initial sequencing, these two samples belong to the lineage B.1.177. None of the products were able to detect one sample, this result may indicate that SARS-CoV-2 RNA degradation has occurred due to freeze-thaw cycles. It was decided to eliminate this sample from the study.

The presence and the absence of the HV 69-70 deletion was analysed. The presence of the HV 69-70 deletion was correctly detected in the 50 samples classified as B.1.1.7 (Table 19). On the other hand, the absence of the HV 69-70 deletion was correctly detected in 48 of the 49 clinical samples with lineages other than B.1.1.7 (Table 20). Regarding the 2 samples with no amplification of the *S* gene with the TaqPath COVID-19, that present the HV 69-70 deletion but confirmed as non-B.1.1.7 by sequencing, the VIASURE assay detect the deletion only in one of them. This result was confirmed by sequencing.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit	**Sequencing results**		
	+	**-**	**Total**
+	50	1*	51
-	0	48	48
Total	50	49	99

Table 19. VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit results compared to sequencing results for the **presence of the HV 69-70 deletion**.

* The presence of HV 69-70 deletion was confirmed by secuencing.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit	Sequencing results		
	+	-	Total
+	48	0	48
-	1*	50	50
Total	49	50	99

Table 20. VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit results compared to TaqPath COVID-19 and sequencing for the **absence of the HV 69-70 deletion**.

* The presence of HV 69-70 deletion was confirmed by secuencing.

Pathogen	Overall agreement	TP	TN	FP	FN	SE	SP	PPV	NPV
UK variant	>99%	50	49	0	0	1 (0.93-1)	1 (0.92-1)	1 (0.93-1)	1 (0.92-1)
Non-UK variant	>99%	49	50	0	0	1 (0.92-1)	1 (0.93-1)	1 (0.92-1)	1 (0.93-1)

Table 21. True positive and negative values, false positive and negative values, sensitivity, specificity, PPV, NPV values for VIASURE *SARS-CoV-2 UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit compared to reference assay.

In this evaluation it was possible to analyse 99 nasopharyngeal clinical samples previously characterized with a reference molecular assay (TaqPath COVID-19 CE-IVD RT-PCR Kit). In the study it was included 2 groups of samples. A group characterized as SARS-CoV-2 non-UK variant and a group characterized as SARS-COV-2 B.1.1.7. All lineages confirmed by sequencing.

Regarding the VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit, VIASURE assays showed an overall agreement of 98.9% with the TaqPath COVID-19 CE-IVD RT-PCR Kit. Incongruent result was obtained in one sample and after sequencing it was verified that VIASURE assay reported the true values.

With this evaluation, it was possible to observe that the VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit proved to be as sensitive and more specific than the reference assay. It has proven to be a good tool for the diagnostic of SARS-CoV-2 and the differentiation of the variants with or without the HV 69-70 deletion being the B.1.1.7 one of the variants with this deletion among the worldwide circulating SARS-CoV-2 strains.

For more details, please see individual Report: "Evaluation of the correct detection of the presence or absence of the 69-70 deletion in the S gene of SARS-CoV-2 in different lineages with the VIASURE real-time PCR assays aimed at detecting SARS-CoV-2 RNA in clinical samples." named file: **VS-EV B117 variant 0321.**

To sum up, the clinical performance of "VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit" has been analyzed through external evaluations that has been conducted in collaboration "Servicio de Microbiología" of Fundación Jiménez Díaz, Madrid and "Servicio de Microbiología" of the Hospital Universitario Marqués de Valdecilla, Santander.

Results show high agreement to detect SARS-CoV-2 and HV 69/70 deletion using VIASURE *SARS-CoV-2 & UK variant (Suk, ORF1ab and N genes)* Real Time PCR Detection Kit.

14 EXTERNAL QUALITY ASSESSMENT PROGRAMS

CerTest Biotect usually participates in the external quality assessment (EQA) programs of different 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 del 69/70, ORF1ab & N genes* 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 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 SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System 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 22. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
INSTAND EQA Program April 2020 "Virus Genome Detection – SARS-CoV-2 panel"					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
340059	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340060	SARS-CoV-2 negative (HCoV OC43 positive)	Negative	Negative	Negative	Negative
340061	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340062	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
340063	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340064	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340065	SARS-CoV-2 negative (HCoV 229E positive)	Negative	Negative	Negative	Negative

Table 22. INSTAND EQA Program April 2020 "Virus Genome Detection – SARS-CoV-2 panel".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **INSTAND EQA Program June/July 2020 "Virus Genome Detection – SARS-CoV-2 panel** that consists of 9 lyophilized specimens (derive from lysates of cells which have been infected with coronavirus). Samples positive for SARS-CoV-2 and samples positive for MERS coronavirus contain heat inactivated virus. Samples positive for other human coronaviruses are not heat inactivated.

The EQA sample panels was extracted with MagDEA Dx SV kit (Batch nº17M020, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. 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 23. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
INSTAND EQA Program June/July 2020 "Virus Genome Detection – SARS-CoV-2 panel"					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
340066	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340067	SARS-CoV-2 negative (HCoV MERS positive)	Negative	Negative	Negative	Negative
340068	SARS-CoV-2 negative (HCoV 229E positive)	Negative	Negative	Negative	Negative
340069	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340070	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
340071	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340072	SARS-CoV-2 negative (HCoV NL63 positive)	Negative	Negative	Negative	Negative
340073	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340074	SARS-CoV-2 negative (HCoV OC43 positive)	Negative	Negative	Negative	Negative

Table 23. INSTAND EQA Program June/July 2020 "Virus Genome Detection – SARS-CoV-2 panel".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **INSTAND EQA Program November 2020 "Virus Genome Detection – SARS-CoV-2 panel** that consists of 7 lyophilized specimens (derive from lysates of cells which have been infected with coronavirus). Samples positive for SARS-CoV-2 and samples positive for MERS coronavirus contain heat inactivated virus. Samples positive for other human coronaviruses are not heat inactivated. The EQA sample panels were extracted with MagDEA Dx SV kit (Batch n°22M031, expiry date 2022-05), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n° SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. 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 24. Please, you should consider that we had not participated officially with this product "*SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
INSTAND EQA Program November 2020 "Virus Genome Detection – SARS-CoV-2 panel"					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
340075	SARS-CoV-2 positive (BetaCoV/Berlin/ChVir1670/2020_IsolatBER)	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340076	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
340077	SARS-CoV-2 positive (BetaCoV/Munich/ChVir984/2020)	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340078	SARS-CoV-2 negative (HCoV 229E positive)	Negative	Negative	Negative	Negative
340079	SARS-CoV-2 positive (BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER)	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340080	SARS-CoV-2 positive (BetaCoV/Munich/ChVir984/2020)	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
340081	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant

Table 24. INSTAND EQA Program November 2020 "Virus Genome Detection – SARS-CoV-2 panel".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **INSTAND EQA Program November 2020 "Virus Genome Detection – Respiratory Virus Panel 2 for Multiplex Tests** that consists of 4 lyophilized specimens (lyophilized cell lysates or allantoic fluid of infected embryonated chicken eggs or suspension of nasopharyngeal swabs). Samples positive for MERS coronavirus and samples positive for SARS-CoV-2 are heat inactivated. The samples containing avian influenza A virus are chemically inactivated. The EQA sample panels were extracted with MagDEA Dx SV kit (Batch n°23M020, expiry date 2022-10), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n° SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. 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 25. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
INSTAND EQA Program November 2020 "Virus Genome Detection – Respiratory Virus Panel 2 for Multiplex Tests"					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
432013	**Influenza B virus** B/Berlin/16/2020 (B/Victoria-line), (B/Washington/02/2019-like)	Negative	Negative	Negative	Negative
432014	**Influenza A virus (H1N1)pdm09 + RSV B** A/Sachsen Anhalt/20/2020 (H1N1)pdm09 (A/Hawaii/70/2019-like)	Negative	Negative	Negative	Negative
432015	Negative	Negative	Negative	Negative	Negative
432016	**Influenza B virus** B/Phuket/3073/2013-like (B/Yamagata-line) **+ RSV A/ON1** + **SARS-CoV-2** (BetaCoV/Munich/ ChVir984/2020)	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant

Table 25. INSTAND EQA Program November 2020 "Virus Genome Detection – Respiratory Virus Panel 2 for Multiplex Tests".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* 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 nº98M030, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. 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 26. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
QCMD 2020 Coronavirus Outbreak Preparedness EQA Pilot Study					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
CVOP20S-01	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
CVOP20S-02	SARS-CoV-2 negative (HCoV NL63 positive)	Negative	Negative	Negative	Negative
CVOP20S-03	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
CVOP20S-04	SARS-CoV-2 negative (HCoV OC43 positive)	Negative	Negative	Negative	Negative
CVOP20S-05	Negative	Negative	Negative	Negative	Negative
CVOP20S-06	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
CVOP20S-07	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
CVOP20S-08	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant

Table 26. QCMD 2020 Coronavirus Outbreak Preparedness EQA Pilot Study.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **QCMD 2020 Coronavirus (RNA) EQA Programme** (CVRNA101S). The EQA sample panels 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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n° SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. 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 27. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
QCMD 2020 Coronavirus (RNA) EQA Programme					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
CVRNA101S-01	Coronavirus OC43	Negative	Negative	Negative	Negative
CVRNA101S-02	Coronavirus NL63	Negative	Negative	Negative	Negative
CVRNA101S-03	Coronavirus 229E	Negative	Negative	Negative	Negative
CVRNA101S-04	Coronavirus OC43	Negative	Negative	Negative	Negative
CVRNA101S-05	Coronavirus Negative	Negative	Negative	Negative	Negative
CVRNA101S-06	Coronavirus HKU	Negative	Negative	Negative	Negative
CVRNA101S-07	Coronavirus NL63	Negative	Negative	Negative	Negative
CVRNA101S-08	Coronavirus NL63	Negative	Negative	Negative	Negative

Table 27. QCMD 2020 Coronavirus (RNA) EQA Programme.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **QCMD 2020 MERS Coronavirus EQA Programme** (MERS101S). The EQA sample panels 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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n° SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. The panel consist of 8 frozen transport medium samples with various concentrations of MERS Coronavirus or samples negative for MERS 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 28. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
QCMD 2020 MERS Coronavirus EQA Programme					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
MERS101S-01	MERS Coronavirus	Negative	Negative	Negative	Negative
MERS101S-02	MERS Coronavirus	Negative	Negative	Negative	Negative
MERS101S-03	Coronavirus OC43	Negative	Negative	Negative	Negative
MERS101S-04	MERS Coronavirus	Negative	Negative	Negative	Negative
MERS101S-05	Coronavirus Negative	Negative	Negative	Negative	Negative
MERS101S-06	Coronavirus NL63	Negative	Negative	Negative	Negative
MERS101S-07	MERS Coronavirus	Negative	Negative	Negative	Negative
MERS101S-08	MERS Coronavirus	Negative	Negative	Negative	Negative

Table 28. QCMD 2020 MERS Coronavirus EQA Programme.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **QCMD 2020 SARS-CoV-2 EQA Programme** (SCV2_101S). The EQA sample panels were extracted with MagDEA Dx SV kit (Batch n°22M031, expiry date 2022-11), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n° SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. The panel consist of 10 frozen transport medium samples with various concentrations of SARS-CoV-2 or samples negative for SARS-CoV-2. 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 29. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
QCMD 2020 SARS-CoV-2 EQA Programme					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
SCV2_101S-01	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
SCV2_101S-02	SARS-CoV-2 negative (HCoV 229E positive)	Negative	Negative	Negative	Negative
SCV2_101S-03	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
SCV2_101S-04	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
SCV2_101S-05	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
SCV2_101S-06	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
SCV2_101S-07	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
SCV2_101S-08	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
SCV2_101S-09	Coronavirus Negative	Negative	Negative	Negative	Negative
SCV2_101S-10	SARS-CoV-2 negative (HCoV OC43 positive)	Negative	Negative	Negative	Negative

Table 29. QCMD 2020 SARS-CoV-2 EQA Programme.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **QCMD 2020 Respiratory I Plus EQA Programme** (RESPIplus101S). The EQA sample panels were extracted with MagDEA Dx SV kit (Batch n°23M020, expiry date 2022-10), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n° SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. The panel consist of 10 frozen transport medium samples with various concentrations of Influenza virus A or B, respiratory syncytial virus or SARS-CoV-2 or samples negative for Influenza virus A & B, respiratory syncytial virus and SARS-CoV-2. 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 30. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
QCMD 2020 Respiratory I Plus EQA Programme					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
RESPIplus101S-01	Influenza Type A (H1N1)pdm09	Negative	Negative	Negative	Negative
RESPIplus101S-02	Influenza Type A (H3N2)	Negative	Negative	Negative	Negative
RESPIplus101S-03	Influenza Type B (Victoria)	Negative	Negative	Negative	Negative
RESPIplus101S-04	Influenza Type B (Yamagata)	Negative	Negative	Negative	Negative
RESPIplus101S-05	RSV Type A	Negative	Negative	Negative	Negative
RESPIplus101S-06	RSV Type B	Negative	Negative	Negative	Negative
RESPIplus101S-07	SARS-CoV-2	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
RESPIplus101S-08	SARS-CoV-2	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
RESPIplus101S-09	Influenza Type A (H1N1) pdm09 + SARS-CoV-2	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
RESPIplus101S-10	Negative	Negative	Negative	Negative	Negative

Table 30. QCMD 2020 Respiratory I Plus EQA Programme.

VIASURE kit was evaluated in our facilities with the **NEQAS for Microbiology**, **"Molecular detection of SARS-CoV-2"** (August 2020 - Distribution: 4886, August 2020 - Distribution: 4887, September 2020 - Distribution: 4889, October 2020 - Distribution: 4890 and November 2020 - Distribution: 4891). The panels of specimens were extracted with MagDEA Dx SV kit (Batches n°18M020, 20M020 and 17M020, expiry dates 2022-05, 2022-05 and 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.)); and analysed with VIASURE assay (Batch n° SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™Real-Time PCR Detection System. 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 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* 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 – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
6333	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
6334	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
August 2020 - Distribution: 4887					
Sample code	**Sample composition**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
6335	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
6336	SARS-CoV-2 negative (Influenza B positive)	Negative	Negative	Negative	Negative
September 2020 - Distribution: 4889					
Sample code	**Sample composition**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
6352	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
6353	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
October 2020 - Distribution: 4890					
Sample code	**Sample composition**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
6354	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
6355	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
November 2020 - Distribution: 4891					
Sample code	**Sample composition**	**Viasure – HV 69/70 deletion**	**Viasure – *ORF1ab* gene**	**Viasure – *N* gene**	**Viasure – Final Result**
6356	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
6357	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant

Table 31. NEQAS for Microbiology 2020/2021 - Molecular detection of SARS-CoV-2 results.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit was evaluated with **LGC Standards Proficiency Testing 2020** (COV-SARS-CoV-2 molecular, CLCV001, Round 1, 27 May 2020, COV-SARS-CoV-2 molecular, CLCV002, Round 2, 14 Sep 2020, and COV-SARS-CoV-2 molecular, CLCV003, Round

3, 16 Nov 2020). The EQA sample panels were extracted with MagDEA Dx SV kit (Batches nº98M030 and 22M031, expiry dates 2020-09 and 2022-05), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™Real-Time PCR Detection System. 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 32. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
LGC Standards Proficiency Testing 2020. COV-SARS-CoV-2 molecular, CLCV001, Round 1, 27 May 2020.					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *N2 target***	**Viasure – *N1 target***	**Viasure – Final Result**
COV-A	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
COV-B	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
LGC Standards Proficiency Testing 2020. COV-SARS-CoV-2 molecular, CLCV002, Round 2, 14 Sep 2020.					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – N2 target**	**Viasure – N1 target**	**Viasure – Final Result**
COV-A	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
COV-B	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
LGC Standards Proficiency Testing 2020. COV-SARS-CoV-2 molecular, CLCV003, Round 3, 16 Nov 2020.					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – N2 target**	**Viasure – N1 target**	**Viasure – Final Result**
COV-A	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
COV-B	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant

Table 32. LGC Standards Proficiency Testing 2020.

The VIASURE kit was evaluated in our facilities with **CAP SARS-CoV-2 Molecular Proficiency Testing Program COV2A-2020 and COV2B-2020.** These panels consist of 6 non-infectious liquid specimens. The EQA sample panels were extracted with MagDEA Dx SV kit (Batch nº98M030 and 23M020, expiry dates 2020-09 and 2022-10), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº SUK2XL-EXP.517B, expiry

date 2023-02) on Bio-Rad CFX96™Real-Time PCR Detection System. 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 33. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
CAP SARS-CoV-2 Molecular Proficiency Testing Program COV2A-2020 and COV2B-2020					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *N2 target***	**Viasure – *N1 target***	**Viasure – Final Result**
COV2-01	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
COV2-02	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
COV2-03	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
COV2-04	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
COV2-05	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant
COV2-06	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant

Table 33. CAP SARS-CoV-2 Molecular Proficiency Testing Program COV2A-2020 and COV2B-2020.

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* 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 nº98M030, expiry date 2020-09), using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº SUK2XL-EXP.517B, expiry date 2023-02) on Bio-Rad CFX96™ Real-Time PCR Detection System. 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 34. Please, you should consider that we had not participated officially with this product "VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit".

VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit					
LABQUALITY SARS-CoV-2, nucleic acid detection. Round 1, 2020 (Pilot)					
Sample code	**Sample source**	**Viasure – HV 69/70 deletion**	**Viasure – *N2 target***	**Viasure – *N1 target***	**Viasure – Final Result**
S003 (LQ775720013)	SARS-CoV-2 negative	Negative	Negative	Negative	Negative
S004 (LQ775720014)	SARS-CoV-2 positive	Negative	Positive	Positive	Positive SARS-CoV-2 non-UK variant

Table 34. LABQUALITY SARS-CoV-2, nucleic acid detection. Round 1, 2020 (Pilot).

To sum up, 95 specimens from EQA programmes (**45/95 positive samples for SARS-CoV-2 non-UK variant**) were found positive with VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit.

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 the online resource Westgard QC, Inc. The results are described below in Table 35.

	VIASURE VALUES in EQA programmes from 2020	
	SARS-CoV-2 non-UK variant	SARS-CoV-2 UK variant
PPA	100.0% (92.1%- 100.0%)	-
NPA	100.0% (92.9%- 100.0%)	100.0% (96.1%- 100.0%)
OPA	100.0% (96.1%- 100.0%)	100.0% (96.1%- 100.0%)

Table 35. 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 UK variant using VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* 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 SUK2". The results of programs that we had participated have been recorded internally on Excel data sheets "EQA Programme Results".

15 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%).

Since the initial genomic characterization of SARS-CoV-2, the virus has been divided into different genetic groups or clusters (S, L, V, G with GH and GR subgroups). The appearance of mutations is a natural and expected event within the evolution process of the virus. In fact, some specific mutations define the viral genetic groups that are currently circulating globally. The mutations identified to date remain within the expected patterns for a coronavirus. Viruses classified in genetic group G are the most frequent worldwide. Thanks to the genetic sequencing of the pathogen worldwide, it has been possible to establish patterns of dispersal and evolution of the virus.

On December 14, 2020, the United Kingdom declared an increase in the incidence of SARS-CoV-2 in some regions of its country associated with a new variant of the virus with a supposed greater transmission capacity. This variant, called VOC202012/01 (B.1.1.7) presented 23 different mutations: 13 non-synonymous, including a series of mutations in the spike protein (S), 4 deletions and 6 synonymous. By the end of December, this variant had been detected in 31 countries and territories in 5 of the 6 WHO regions. One of the mutations is the deletion at positions 69-70 in the spike protein. Detection of the HV 69/70 deletion is of great importance since it has been related to immune leakage in immunosuppressed patients and to increased viral infectivity. Another cause for concern in relation to the HV 69/70 deletion is that it affects the sensitivity of virus detection using molecular techniques (RT-PCR) that detects the *S* gene.

The deletion H69/V70 is present in over 6000 sequences worldwide, 2.5% of the available, and largely in Europe from where most of the sequences in GISAID are derived. Many of the sequences are from the UK and Denmark where sequencing rates are high compared to other countries. ΔH69/V70 occurs in variants observed in different global lineages, representing multiple independent acquisitions of this SARS-CoV-2 deletion. While variants with deletions in this region of Spike are observed in GISAID, the earliest unambiguous sequence that includes the ΔH69/V70 was detected in Sweden in April 2020, an independent deletion event relative to other ΔH69/V70 variants. The prevalence of ΔH69/V70 has since increased since August 2020.

The presence of the HV 69/70 deletion is associated with the UK variant, lineage B.1.1.7, however, other variants such as B.1.1.298 (Danish lineage) or B.1.258 also have this deletion. In January 2021, scientists from UK reported evidence that suggests the B.1.1.7 variant may be associated with an increased risk of death compared with other variants.

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) and a saliva 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 (two 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.

The appearance of variants that increase the transmissibility of the virus, its virulence or that escape the action of the neutralizing antibodies generated after natural infection or the vaccine, constitute a first-order public health problem that can have an important impact on control of the pandemic. A concern regarding the new variants is that their detection by molecular techniques (RT-PCR) could be affected. For this reason, most commercial PCR tests use multiple targets located on different SARS-CoV-2 genes, so the likelihood that new variants will not be detected decreased.

Early reports found no evidence to suggest that the variant has any impact on the severity of disease or vaccine efficacy.

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16 INTERFERENCES AND INHIBITORS OF PCR

See Interferences and Inhibitors of PCR report.

17 STABILITY ASSAY

The aim of the stability assays is to demonstrate that the characteristics and performance of the **VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* 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.

The results obtained during the stability studies 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 establish 24 months as Product Shelf-life for DNA/RNA kits at temperature range from +2°C to +40°C since manufacturing date.

18 VALIDATION PROCEDURE

This section provides a summary of information and evidence supporting the validity of the assay procedure in terms of important of important reaction conditions during the following steps: Sample Preparation, Nucleic Acid extraction, Reconstitution of the lyophilized Positive Control and Extraction Control (if available), and PCR protocol, Results Interpretation)

The performance characteristics results for: analytical sensitivity, precision (intra-, inter-assays and inter-batch), linearity, analytical specificity and reactivity that have been assessed during the validation of this device whilst following the usage instructions (Sample Preparation, RNA extraction, Reconstitution of the lyophilized Positive Control, and PCR protocol) have been reported in this document.

Besides that, VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* 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 NEOS-96 qPCR (Linear Chemicals).

The panel samples and controls tested on the VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit were previously described in section 4. The arithmetic mean ($\bar{x}$), the standard deviation (σ) and the coefficient of variation (CV%) were calculated and are shown in Tables 36, 37, and 38.

Sample	Target	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	34.36	0.48	1.39
	SARS-CoV-2 (*ORF1ab* gene)	ROX	30.44	0.08	0.25
	SARS-CoV-2 (*N* gene)	Cy5	33.03	0.21	0.63
Three targets positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	34.96	0.94	2.70
	SARS-CoV-2 (*ORF1ab* gene)	ROX	32.00	0.47	1.46
	SARS-CoV-2 (*N* gene)	Cy5	33.61	1.52	4.53
Negative sample	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	23.60	0.34	1.43
Positive Control	SARS-CoV-2 (HV 69/70 deletion)	FAM	24.18	0.02	0.06
	SARS-CoV-2 (*ORF1ab* gene)	ROX	25.12	0.02	0.07
	SARS-CoV-2 (*N* gene)	Cy5	22.16	0.06	0.25
Negative Control	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 36. VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02) assay run on the **Bio-Rad CFX96 TouchTM Real-Time PCR Detection System**. (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct va value, (**σ**) = standard deviation, (**CV %**) = coefficient of variation, Neg = negative, n.a. = not applicable.

Sample	Target	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	33.14	0.24	0.71
	SARS-CoV-2 (*ORF1ab* gene)	ROX	30.22	0.18	0.58
	SARS-CoV-2 (*N* gene)	Cy5	31.04	0.10	0.32
Three targets positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	33.98	0.65	1.92
	SARS-CoV-2 (*ORF1ab* gene)	ROX	32.25	0.92	2.85
	SARS-CoV-2 (*N* gene)	Cy5	31.64	1.12	3.54
Negative sample	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	23.24	0.19	0.84
Positive Control	SARS-CoV-2 (HV 69/70 deletion)	FAM	23.56	0.06	0.23
	SARS-CoV-2 (*ORF1ab* gene)	ROX	24.34	0.09	0.37
	SARS-CoV-2 (*N* gene)	Cy5	20.60	0.14	0.70
Negative Control	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 37. VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch n°: SUK2XL-EXP.517B, expiry date 2023-02) 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	Target	Viasure channel	$\bar{x}$ (Ct)	σ	CV%
Positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	30.34	0.24	0.79
	SARS-CoV-2 (*ORF1ab* gene)	ROX	28.45	0.38	1.32
	SARS-CoV-2 (*N* gene)	Cy5	30.85	0.63	2.05
Three targets positive	SARS-CoV-2 (HV 69/70 deletion)	FAM	32.20	0.77	2.38
	SARS-CoV-2 (*ORF1ab* gene)	ROX	28.97	0.35	1.22
	SARS-CoV-2 (*N* gene)	Cy5	31.91	0.28	0.88
Negative sample	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	22.58	0.03	0.14
Positive Control	SARS-CoV-2 (HV 69/70 deletion)	FAM	22.55	0.70	3.08
	SARS-CoV-2 (*ORF1ab* gene)	ROX	22.98	0.12	0.51
	SARS-CoV-2 (*N* gene)	Cy5	20.70	0.11	0.53
Negative Control	SARS-CoV-2 (HV 69/70 deletion, *ORF1ab* and *N* genes)	FAM/ROX/Cy5	Neg	n.a.	n.a.
	Endogenous Internal Control	HEX	Neg	n.a.	n.a.

Table 38. VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit (Batch nº: SUK2XL-EXP.517B, expiry date 2023-02) assay run on the **NEOS-96 qPCR (Linear Chemicals).** (**Ct**) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (**σ**) = standard d deviation, (**CV** %) = coefficient of variation, Neg = negative, n.a. = not applicable.

The results have been recorded internally on Excel data sheet "2 Precision SUK2".

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 del 69/70, ORF1ab & N genes* 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.

19 VIASURE SCIENTIFIC REFERENCE

1. V. Pérez, B. Dehesa, C. Escolar, L. Llobet & H. Alonso. Identificación de la variante SARS-CoV-2 B.1.1.7 (variante británica) por la ausencia de amplificación del gen S: ¿Es un buen método de diagnóstico clínico? XXIV Congreso Nacional de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC) 2021.
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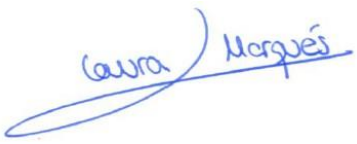
In conclusion, we consider that the VIASURE *SARS-CoV-2 del 69/70, ORF1ab & N genes* Real Time PCR Detection Kit is suitable for its intended use and performs accordingly to the pre-determined criteria.

Change Control		
Version No.	**Changes**	**Date**
00	Original Version	09/07/2021

Table 39. Control change table.

Name	Laura Marqués Gracia	Leyre Vicente-Ginés
Signature		
Function	*Molecular Diagnostic Regulatory Affairs Technician*	*Molecular Diagnostic Regulatory Affairs Manager*

DATE: (dd/mm/yyyy) **09/07/2021**

Revision Code: rev.00

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