VIASURE

Real Time PCR Detection Kit

bestbion dx

smarter diagnostics

# SARS-CoV-2 Variant I

CE IVD

These instructions for use apply to the following references / *Estas instrucciones de uso aplican para las siguientes referencias:*

**OPEN FORMAT (SEE ANNEX 1) / *OPEN FORMAT (VER ANEXO 1)***

| PRODUCT / PRODUCTO | REFERENCE / REFERENCIAS |
|---|---|
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 6 x 8-well strips, low profile | VS-VAR106L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 6 x 8-well strips, high profile | VS-VAR106H |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 12 x 8-well strips, low profile | VS-VAR112L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 12 x 8-well strips, high profile | VS-VAR112H |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 96-well plate, low profile | VS-VAR113L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 96-well plate, high profile | VS-VAR113H |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 1 x 8-well strips, low profile | VS-VAR101L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 1 x 8-well strips, high profile | VS-VAR101H |

**Table A 1. References for Open format products. / *Referencias para productos Open Format.***

**TUBE FORMAT (SEE ANNEX 2) / *FORMATO TUBO (VER ANEXO 2)***

| PRODUCT / PRODUCTO | REFERENCE / REFERENCIAS |
|---|---|
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit, 4 tubes x 24 reactions | VS-VAR196T |

**Table A 2. References for Tube format control products. / *Referencias para productos formato Tubo.***

# Content

ENGLISH

# 1. Intended use

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit is a real-time RT-PCR test designed for the qualitative detection of RNA from genetic mutations in the S gene (E484K, K417N, K417T and N501Y) from positive SARS-CoV-2 nasopharyngeal samples This test is intended for use as an aid to monitor the prevalence of genetic mutations in the S gene (E484K, K417N, K417T and N501Y) and to assist in control measures. RNA is extracted from respiratory specimens. Complementary DNA (cDNA) is synthetised and amplified using RT-PCR and detected using fluorescent reporter dye probes specific for genetic mutations in the S gene (E484K, K417N, K417T and N501Y).

# 2. Summary and Explanation

The SARS-CoV-2 virus was detected for the first time in Wuhan (China) on December 31th, 2019. During 2020, the virus spread progressively throughout the world and on March 11th, the WHO decreed the state of pandemic.

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. 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. This variant has a mutation in the receptor binding domain (RBD) of the spike protein at position 501, where the amino acid asparagine (N) has been replaced with tyrosine (Y), called N501Y. Other characteristically mutation of this variant is the 69/70 deletion or P681H.

B.1.351 variant, first identified in Nelson Mandela Bay, South Africa, in samples dating back to the beginning of October 2020. The variant also was identified in Zambia in late December 2020, at which time it appeared to be the predominant variant in the country. This variant has multiple mutations in the spike protein, including K417N, E484K, N501Y. It has potential reduction in neutralization by some EUA monoclonal antibody treatments.

The SARS-CoV-2 epidemic in Brazil was dominated by two lineages designated as P.1 and P.2, harboring mutations at the receptor-binding domain of the Spike (S) protein. Lineage P.1 (referred as B.1.1.28) is considered a Variant of Concern (VOC) because it has potential reduction in neutralization by some EUA monoclonal antibody treatments. This Lineage presents multiple mutations in the S protein (including K417T, E484K, N501Y) and its emergence was associated with a second COVID-19 epidemic wave in the Amazonas state. Lineage P.2 (referred as B.1.1.33) is considered a Variant Under Investigation (VUI) and only harbors the mutation E484K. The P.2 lineage has been detected as the most prevalent variant in several states across the country in late 2020 and early 2021.

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, VIASURE SARS-CoV-2

Variant I Real Time PCR Detection Kit allows the detection of the main mutation associated with the variant under surveillance.

## 3. Principle of the procedure

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit is designed for the qualitative detection of RNA from genetic mutations in the S gene (E484K, K417N, K417T and N501Y) from positive SARS-CoV-2 respiratory samples. The detection is done in one step real time RT format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction well. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase which is followed by the amplification of a conserved region of the *S* gene for SARS-CoV-2 E484K, K417N, K417T and N501Y using specific primers and a fluorescent-labeled probe.

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit is based on the 5´ exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bounded to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of target template. This fluorescence can be measured on Real Time PCR platforms.

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit contains in each well all the components necessary for real time PCR assay (specific primers/probes, dNTPS, buffer, polymerase and retrotranscriptase) in a stabilized format.

## 4. Reagents provided

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit includes the materials and reagents detailed in Annex 1 for open format products and Annex 2 for tube format products.

## 5. Reagents and equipment to be supplied by the user

The following list includes the materials that are required for use but not included in the VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit.

- Real Time PCR instrument (thermocycler).
- Real Time PCR compatible plastic consumables (i.e. individual tubes, well-strips and/or microplates). Only for Tubes format (Annex 2).
- RNA extraction kit.
- Collection and transport system.
- Laboratory freezers: - 30°C to - 10°C and/or ≤ –70°C.
- Centrifuge for 1.5 mL tubes and PCR-well strips or 96-well plate (if available).
- Vortex.
- Micropipettes (0.5-20 µL, 20-200 µL).
- Filter tips.
- Powder-free disposable gloves.

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit has been validated on the following 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, Roche LightCycler 480 Instrument, Linear NEOS-96 Real Time PCR System and Rotor-Gene® Q. When using the Applied Biosystems 7500 Fast with strips it is recommend to place a plate holder to reduce the risk of crushed tube (Ref. PN 4388506). To check thermocycler compatibility and most common detection channels consult website ( www.certest.es).

Optical measurement parameters of some thermocyclers must be adjusted to be suitable for operation with "VIASURE Real Time PCR Detection Kits". This assay has been validated with the following set exposition values:

- DTprime Real-time Detection Thermal Cycler (DNA-Technology): FAM channel -500*, HEX channel – 500, ROX channel – 1000 and Cy5 channel - 1000.

- DTlite Real-Time PCR System (DNA-Technology): FAM channel - 500, HEX channel - 250, ROX channel – 500 and Cy5 channel - 500.

*If the result in channel FAM is not as expected, there are no amplifications or high background noise is observed, please lower the exposure values indicated above to 150.

## 6. Transport and storage conditions

- The kits can be shipped and stored at 2-40°C until the expiration date which is stated on the label.
- Once the positive control has been re-suspended, store it at -20°C. It is recommended to separate it in aliquots to minimize freeze and thaw cycles. Positive control has been validated as still being stable after 6 freeze-thaw cycles.
- Keep components away from light.
- For Tube format kits: Once the *SARS-CoV-2 Variant I* Reaction-Mix tube has been reconstituted, it may be kept it at 25°C±5°C or 2°C to 8°C for up to 4 hours. For a longer period of time, it is recommended store at –20°C and to separate in aliquots to minimize freeze and thaw cycles (up to 6 times).

## 7. Precautions for users

- The product is indented for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures (including training on the Real Time PCR instrument (thermocycler) and Nucleic acid extraction system).
- For *in vitro* diagnostic use.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective box is open or broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches once is open.
- Close protective pouches of reagents promptly with the zip seal after each use (for references: VS-VAR113L, and VS-VAR113H). Remove any air excess in the pouches prior to closing.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and / or kits and / or lots and / or another supplier.
- Protect reagents against from humidity. Prolonged exposure to humidity may affect product performance.

- An appearance of the reaction mixture in stabilized format, normally found at the bottom of the tube, different from the usual one (without conical shape, inhomogeneous, smaller/larger in size and/or color different from whitish) does not alter the functionality of the test.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed. Use separate areas for the preparation of patient samples and controls to prevent false positive results.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit and any additional reagents or equipment required for testing are not contaminated. Always avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents at all times. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles and mask. Do not eat, drink, smoke or apply cosmetic products in the working area. Wash your hands after finishing the test.
- Specimens must be treated as potentially infectious and/or biohazardous, as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national safety regulations. Take necessary precautions during the collection, transport, storage, handling, and disposal of samples.
- Samples and reagents must be handled in a biological safety cabinet. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Dispose of waste in compliance with local and state regulations.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- In accordance with Regulation (EC) No 1907/2006 (REACH), VIASURE Real Time PCR Detection Kits do not require Material Safety Data Sheets on account of their classification as non-hazardous to health and the environment, because they do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) No 1272/2008 (CLP), or which are in concentrations higher than the value established in the mentioned regulation for their declaration.
- Consult each Real Time PCR instrument's reference manual for additional warnings, precautions and procedures.

# 8. Test procedure

Please see Annex 1 for Open format products Test Procedure and Annex 2 for Tube format products Test Procedure.

## 8.1. Specimen collection, transport and storage

The VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection kit has been tested on nasopharyngeal specimens collected with synthetic fiber swabs with plastic and placed immediately into a sterile transport tube containing Viral transport medium (VTM). Other types of samples must be validated by the user.

Collection, storage, and transport specimens should be maintained per the conditions validated by the user. Overall, respiratory samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type) and processed as soon as possible to guarantee the quality of the

test. 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), It is recommended shipping at -20°C or lower. 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. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

The respiratory specimens must be collected, transport and storage according to appropriate laboratory guidelines. For details, refer to the CDC guideline (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).

## 8.2. RNA extraction

Perform the sample preparation according to the recommendations appearing in the instructions for use of the extraction kit used.

For RNA extraction from respiratory samples, you can use your manual or automatic routine optimized system, or any commercially available RNA extraction kit and follow the manufacturer´s instructions. The following extraction kits have been validated:

- 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).

# 9. Result interpretation

All the result of the test should be evaluated by a healthcare professional in the context of medical history, clinical symptoms, and other diagnostic tests. The analysis of the controls and samples is done by the software of the used real time PCR equipment itself according to manufacturer´s instructions.

**It is recommended to set the threshold values for each channel (target) independently by the end-user.** Use the Positive Control amplification curve as a starting point during the run validation (before than interpretation of patient sample results), in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments may vary due to different signal intensities.

The use of positive and negative controls in each run, validate the reaction by checking the absence of signal in the negative control well and the presence of signal in the positive control well.

For a valid diagnostic test run, the following control conditions must be met:

| Controls | E484K (*S* gene) (FAM) | K417T (*S* gene) (ROX) | K417N (*S* gene) (HEX) | N501Y (S gene) (Cy5) | Interpretation of Controls |
|---|---|---|---|---|---|
| **Positive Control (PC)** | ≤40 | ≤40 | ≤40 | ≤40 | **Valid** |
| **Negative Control (NC)** | ≥40 or no signal | ≥40 or no signal | ≥40 or no signal | ≥40 or no signal | **Valid** |

**Table 1. Expected Performance of Controls**

1 In cases where either or both of the control assays have failed (an amplification signal is observed in the negative control and/or signals absence in the positive control well for any target channel), all results are reported as 'Invalid' and retesting is required.

Assessment of clinical samples test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If one or more controls are not valid, the patient results cannot be interpreted.

For interpretation of patient sample results, use the following table, read and analyze the results:

| E484K (S gene) (FAM) | K417T (S gene) (ROX) | K417N (S gene) (HEX) | N501Y (S gene) (Cy5) | Interpretation for patients' individual samples | |
|---|---|---|---|---|---|
| ≤40 | ≥40 or no signal | ≥40 or no signal | ≥40 or no signal | **Valid** | **E484K mutation detected** |
| ≥40 or no signal | ≤40 | ≥40 or no signal | ≥40 or no signal | **Valid** | **K417T mutation detected** |
| ≥40 or no signal | ≥40 or no signal | ≤40 | ≥40 or no signal | **Valid** | **K417N mutation detected** |
| ≥40 or no signal | ≥40 or no signal | ≥40 or no signal | ≤40 | **Valid** | **N501Y mutation detected** |
| ≤40 | ≤40 | ≥40 or no signal | ≥40 or no signal | **Valid** | **E484K and K417T mutations detected** |
| ≤40 | ≥40 or no signal | ≤40 | ≥40 or no signal | **Valid** | **E484K and K417N mutations detected** |
| ≤40 | ≥40 or no signal | ≥40 or no signal | ≤40 | **Valid** | **E484K and N501Y mutations detected** |
| ≥40 or no signal | ≤40 | ≤40 | ≥40 or no signal | **Valid** | **K417T and K417N mutations detected** |
| ≥40 or no signal | ≤40 | ≥40 or no signal | ≤40 | **Valid** | **K417T and N501Y mutations detected** |
| ≥40 or no signal | ≥40 or no signal | ≤40 | ≤40 | **Valid** | **K417N and N501Y mutations detected** |
| ≤40 | ≤40 | ≤40 | ≥40 or no signal | **Valid** | **E484K, K417T and K417N mutations detected** |
| ≤40 | ≥40 or no signal | ≤40 | ≤40 | **Valid** | **E484K, K147N and N501Y mutations detected** |
| ≥40 or no signal | ≤40 | ≤40 | ≤40 | **Valid** | **K417T, K417N and N501Y mutations detected** |
| ≤40 | ≤40 | ≤40 | ≤40 | **Valid** | **E484K, K417T, K417N and N501Y mutations detected** |
| ≥40 or no signal | ≥40 or no signal | ≥40 or no signal | ≥40 or no signal | **Valid** | **E484K, K417T, K417N and N501Y mutations not Detected** |

Table 2. Interpretation of individual patient sample results. Ct values. no signal = no amplification curve.

Summary of mutations associated with the following lineages present in the most known Variants of Concern (VOC) and Variants under investigation (VUI):

| Lineages | Originally identified in | Mutations in the *S* gene[1] | | | |
|---|---|---|---|---|---|
| | | E484K | K417T | K417N | N501Y |
| **B.1.1.28** | Brazil | X | X | - | X |
| **B.1.1.33** | Brazil | X | - | - | - |
| **B.1.351** | South Africa | X | - | X | X |
| **B.1.1.7** | UK | - | - | - | X |

Table 3. Summary of mutations associated with known Variants of Concern (VOC) and Variants under investigation (VUI).

[1]https://www.gov.uk/government/publications/covid-19-variants-genomically-confirmed-case-numbers/variants-distribution-of-cases-data. (data up to 19 May 2021).

Other variants can present the mutations K417T, K417N, E484K or N501Y because they are not specific for the variants mentioned.

**Final assignment to a lineage must be done by sequencing.**

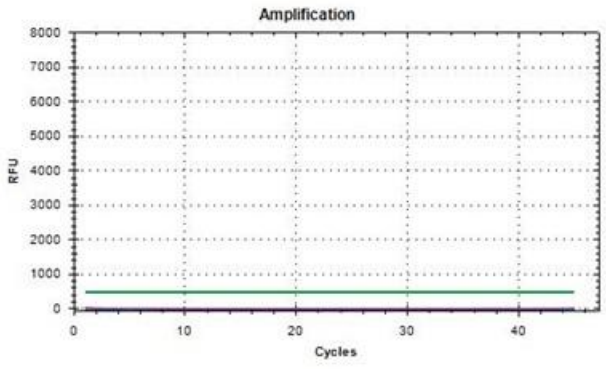
In case of a continued ambiguous result, it is recommended to review the instructions for use; the extraction process used by the user; to verify the correct performance of each RT-qPCR steps and review the parameters; and to check the sigmoid shape of the curve and the intensity of fluorescence. It is also recommended to repeat the assay, preferably in duplicate. Depending on the available material:

a) repeat RT-qPCR with the same isolated RNA sample, or

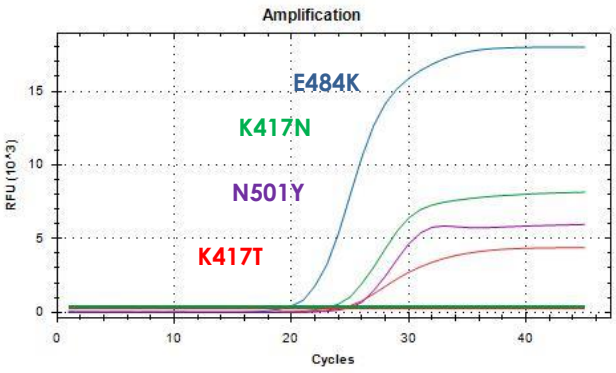
b) re-extract and retest another aliquot of the same specimen or,

c) obtain a new specimen and retest.

Figure 1. Correct run of negative and positive control run on the Bio-Rad CFX96™ Real-Time PCR Detection System.




**Negative control**




**Positive control**

## 10. Limitations of the test

- For professional *in vitro* use.
- Although this assay can be used with other types of samples it has been validated only with RNA extracted from respiratory samples (nasopharyngeal swab).
- The quality of the test depends on the quality of the sample; properly extracted nucleic acid from clinical samples must be extracted.

- This test is a qualitative test and does not provide quantitative values or indicate the number of organisms present.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- There is a possibility of false positive results due to cross-contamination by SARS-CoV-2 RNA from genetic mutations, either the high number of cDNA template copies which contains each *SARS-CoV-2 Variant I* Positive Control vial, samples containing high concentrations of target RNA or contamination due to PCR products from previous reactions.
- False Negative results may arise from several factors and their combinations, including:
    - Improper specimens' collection, transport, storage, and/or handling methods.
    - Improper processing procedures (including RNA extraction).
    - Degradation of the viral RNA during sample shipping/storage and/or processing.
    - Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown SARS-CoV-2 variants.
    - A viral load in the specimen below the limit of detection for the assay.
    - The presence of RT-qPCR inhibitors or other types of interfering substances. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs used to prevent COVID-19 or used during the treatment of the infection have not been evaluated.
    - Failure to follow instructions for use and the assay procedure.

    If in doubt, refer to section 9 to check the correct interpretation of the results.

- A positive test result does not necessarily indicate the presence of viable viruses and does not imply that these viruses are infectious or are the causative agents for clinical symptoms. However, a positive result is indicative of the presence of targets viral sequences.
- The presence of the E484K mutation in the S gene has been first detected in the following lineages: B.1.1.28.1, B.1.1.33, B.1.351, B.1.525, the presence of the K417T mutation in the S gene in the lineage B.1.1.28.1, the presence of the K417N mutation in the S gene in the lineage B.1.351 and the presence of the N501Y mutation in the S gene in the following lineages: B.1.1.28.1, B.1.1.7, B.1.351, however, final assignment to a lineage must be done by sequencing.
- Negative results do not preclude presence of SARS-CoV-2 RNA due to this assay is intended to use with positive SARS-CoV-2 samples.
- Fluorescence values may vary due to multiple factors such as: PCR equipment, extraction system, type of sample, previous treatment of the sample, etc, among others.

## 11. Quality control

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit contains a positive and a negative control that must be included in each run to correctly interpret the results.

# 12. Performance characteristics

## 12.1. Clinical sensitivity and specificity

The clinical performance of VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit was tested using respiratory clinical samples (nasopharyngeal swabs) from patients diagnosed with COVID-19 disease. In order to determine the clinical diagnostic accuracy, different multicenter evaluations have been conducted through collaboration with international entities. A summary of the sites, sample type and workflow is included in the following table:

| | Site | Sample type | Workflow | Target |
|---|---|---|---|---|
| 1 | Addenbrooke's hospital (Cambridge University hospitals NHS Foundation Trust) | nasopharyngeal swab | MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the KingFisher Flex System instrument (ThermoFisher) + Rotor-Gene® Q (QIAGEN) | mutation N501Y |
| | | | | mutation E484K |
| | | | | mutation K417N |
| | | | | mutation K417T |

**Table 4. Site, sample type, workflow and target**

True positive and negative values, false positive and negative values, sensitivity, specificity, PPV, NPV values for VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit were calculated in relation to each comparator assay as shown in the following table:

| Site | Comparator assay | Target | TP | TN | FP | FN | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Whole genome sequencing (WGS) | mutation N501Y | 63 | 25 | 0 | 0 | 1 (0.94-1) | 1 (0.86-1) | 1 (0.94-1) | 1 (0.86-1) |
| | | mutation E484K | 40 | 48 | 0 | 0 | 1 (0.91-1) | 1 (0.92-1) | 1 (0.91-1) | 1 (0.92-1) |
| | | mutation K417N | 20 | 68 | 0 | 0 | 1 (0.83-1) | 1 (0.94-1) | 1 (0.83-1) | 1 (0.94-1) |
| | | mutation K417T | 20 | 68 | 0 | 0 | 1 (0.83-1) | 1 (0.94-1) | 1 (0.83-1) | 1 (0.94-1) |

**Table 5. True positive and negative values, false positive and negative values, sensitivity, specificity, PPV, NPV values for VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit.**

Results show high agreement to detect N501Y, E484K, K417N and K417T mutations using VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit in nasopharyngeal samples previously characterized as SARS-CoV-2 positive.

## 12.2. Analytical sensitivity

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit has a detection limit (LoD) results are as follows:

a) VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit has a detection limit (LoD) of 40 genome copies/rxn for N501Y measured using the SARS-CoV-2 B.1.1.7 lineage and 80 genome copies/rxn measured with RNA from SARS-CoV-2 B.1.351 lineage.

b) VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit has a detection limit (LoD) of 40 genome copies/rxn for E484K measured using RNA from SARS-CoV-2 B.1.351 lineage and SARS-CoV-2 B.1.1.28 lineage.

c) VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit has a detection limit (LoD) of 40 genome copies/rxn for K417N measured using RNA from SARS-CoV-2 B.1.351 lineage.

d) VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit has a detection limit (LoD) of 80 genome copies/rxn for K417T measured using RNA from SARS-CoV-2 B.1.1.28 lineage.

**Figure 2. Dilution series of mutation E484K ($10^7$-$10^1$ copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (FAM channel).**

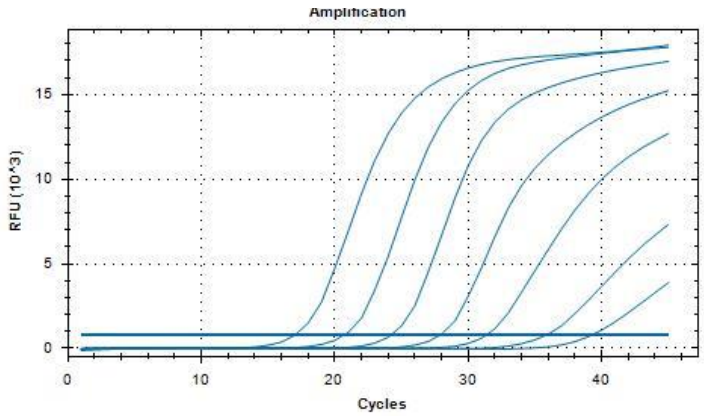



**Figure 3. Dilution series of mutation K417N ($10^7$-$10^1$ copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (HEX channel).**

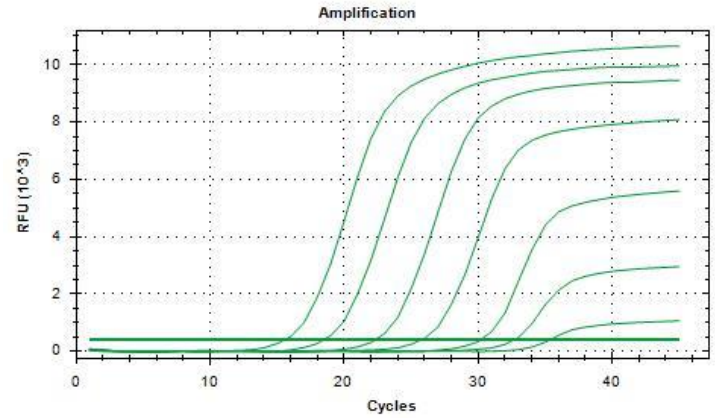



**Figure 4. Dilution series of mutation K417T ($10^7$-$10^1$ copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (ROX channel).**

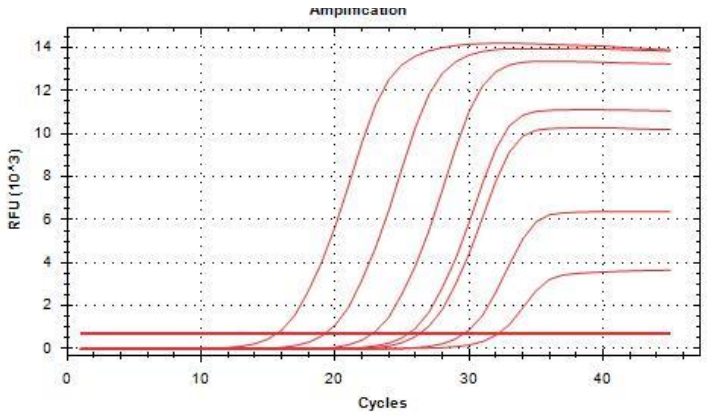
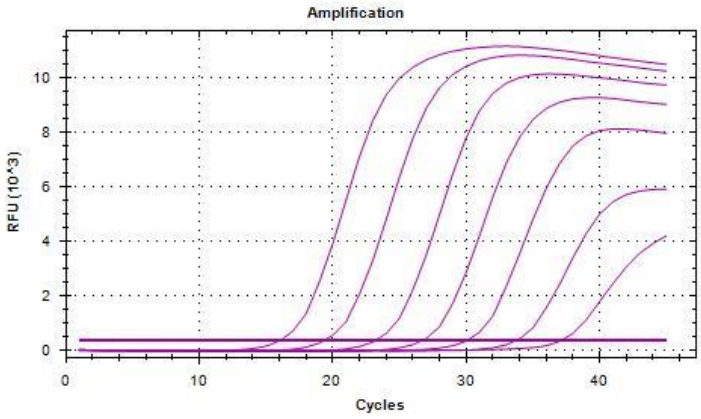


Figure 5. Dilution series of mutation N501Y (107-101 copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (Cy5 channel).




## 12.3. Analytical specificity

The specificity of the SARS-CoV-2 assay was confirmed by testing a panel consisting of different microorganisms representing the most common respiratory pathogens. No cross-reactivity was detected between any of the following microorganisms tested, except the targeted pathogens of each assay.

| Cross-reactivity testing | | | | | |
|---|---|---|---|---|---|
| Human Adenovirus types 1-5, 8, 15, 31, 40 and 41 | - | Influenza A/Victoria/210/2009 (H3N2) | - | Human parainfluenza 1, 2, 3 and 4 viruses | - |
| Human Bocavirus | - | Influenza A/Thüringen/5/17 (H3N2) virus | - | *Pneumocytis jirovecii* Type A1 and g885652 | - |
| *Bordetella bronchiseptica* | - | Influenza A/Switzerland/9715293/2013 (H3N2) virus | - | Human rhinovirus type C | - |
| *Bordetella holmesii* | - | Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus | - | *Staphylococcus aureus* subsp. *aureus* | - |
| *Bordetella parapertussis* | - | Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus | - | *Staphylococcus epidermidis* | - |
| *Bordetella pertussis* | - | Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus | - | *Streptococcus pneumoniae* Z022 | - |
| *Chlamydia caviae* | - | Influenza A/Anhui/1/2013 (H7N9) virus | - | *Streptococcus pyogenes* | - |
| *Chlamydia psittaci* genotype A and C | - | Influenza B/Brisbane/60/2008 | - | *Streptococcus salivarius* | - |
| *Chlamydophila pneumoniae CM-1* | - | Influenza B/Florida/04/06 virus | - | Respiratory syncytial virus (RSV) A and B | - |
| Human coronavirus 229E, OC43, NL63 and HKU1 | - | Influenza B/Phuket/3073/2013 virus | - | SARS Coronavirus Strain Frankfurt 1 | - |
| MERS Coronavirus | - | *Legionella bozemanii* | - | Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1 * | - |
| Enterovirus 68 and 71 | - | *Legionella dumoffii* | - | Human 2019-nCoV strain 2019-nCoV/Italy-INMI1 * | - |
| Enterovirus Echovirus 30 | - | *Legionella longbeachae* | - | SARS-CoV-2 strain 2019nCoV/USA-WA1/2020 * | - |
| Enterovirus Coxsackievirus A24, A9 and B3 | - | *Legionella micdadei* | - | SARS-CoV-2 BetaCoV/Berlin/ChVir1670/2020_IsolatBER* | - |
| *Haemophilus influenzae MinnA* | - | *Legionella pneumophila* | - | SARS-CoV-2 BetaCoV/Munich/ChVir984/2020* | - |
| Influenza A/New Caledonia/20/99(H1N1) virus | - | Human metapneumovirus A and B | - | SARS-CoV-2 BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER* | - |
| Influenza A/California/7/2009(H1N1)pdm09 | - | *Moraxella catarrhalis* | - | MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020) * | - |
| Influenza A/Michigan/45/2015 (H1N1)pdm09 virus | - | *Mycoplasma pneumoniae* | - | MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1) * | - |
| Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus | - | *Mycobacterium tuberculosis* not rifampin resistant | - | | |

**Table 6. Reference pathogenic microorganisms used in this study.**

* Please note that the detection of these SARS-CoV-2 strains is not considered in this assay. This test is designed for the qualitative detection of RNA from specific genetic mutations in the *S* gene (E484K, K417N, K417T and N501Y) present in several SARS-CoV-2 variants.

## 12.4. Analytical reactivity

The reactivity of the VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit for E484K mutation was evaluated against RNA from B.1.351(Twist Synthetic SARS-CoV-2 RNA Control 16) and B.1.1.28 (Twist Synthetic SARS-CoV-2 RNA Control 17), showing positive result.

The reactivity of the VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit for K417N mutation was evaluated against RNA from B.1.351(Twist Synthetic SARS-CoV-2 RNA Control 16), showing positive result.

The reactivity of the VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit for K417T mutation was evaluated against RNA from B.1.1.28 (Twist Synthetic SARS-CoV-2 RNA Control 17), showing positive result.

The reactivity of the VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit for N501Y mutation was evaluated against RNA from B.1.1.7_710528 (Twist Synthetic SARS-CoV-2 RNA Control 14), B.1.1.7_601443 (Twist Synthetic SARS-CoV-2 RNA Control 15), B.1.351(Twist Synthetic SARS-CoV-2 RNA Control 16) and B.1.1.28 (Twist Synthetic SARS-CoV-2 RNA Control 17), showing positive result.

ANNEX 1

# OPEN FORMAT

Annex for the following references:

| PRODUCT | REFERENCE |
|---|---|
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 6 x 8-well strips, low profile | VS-VAR106L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 6 x 8-well strips, high profile | VS-VAR106H |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 12 x 8-well strips, low profile | VS-VAR112L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 12 x 8-well strips, high profile | VS-VAR112H |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 96-well plate, low profile | VS-VAR113L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 96-well plate, high profile | VS-VAR113H |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 1 x 8-well strips, low profile | VS-VAR101L |
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit 1 x 8-well strips, high profile | VS-VAR101H |

**Table A1 1. References**

## A1.1 Principle of the procedure

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit contains in each well all the components necessary for real time PCR assay (specific primers/probes, dNTPS, buffer, polymerase and retrotranscriptase) in a stabilized format.

| Target | Channel | Gene |
|---|---|---|
| E484K | FAM | *S* gene |
| K417N | HEX, VIC or JOE * | *S* gene |
| K417T | ROX | *S* gene |
| N501Y | Cy5 | *S* gene |

**Table A1 2. Target, channel and genes.**

***Depending on the equipment used select the proper detection channel, to check most common detection channels consult the website www.certest.es.**

## A1.2 Reagents provided

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit includes the following materials and reagents detailed in Tables A1.3 and A1.4. Based on the commercial presentation and the Real Time PCR platform used, the stabilized PCR reaction mix could be placed inside different wells and could be marketed on multiple formats. Table A1.3 includes materials and reagents to be used with 8-well strips compatible devices. Table A1.4 includes materials and reagents to be used with 96-well plate compatible devices. (Consult the thermocycler compatibility on CerTest´s website (www.certest.es).

| Reagent/Material | Description | Colour | Amount |
|---|---|---|---|
| *SARS-CoV-2 Variant I* 8-well strips | A mix of enzymes, primers probes, buffer, dNTPs, stabilizers in stabilized format | White | 6/12 x 8-well strip |
| Rehydration Buffer | Solution to reconstitute the stabilized product | Blue | 1 vial x 1.8 mL |
| *SARS-CoV-2 Variant I* Positive Control | Non-infectious synthetic lyophilized cDNA | Red | 1 vial |
| Negative control | Non template control | Violet | 1 vial x 1 mL |
| Water RNAse/DNAse free | RNAse/DNAse free water | White | 1 vial x 1 mL |
| Tear-off 8-cap strips | Optical caps for sealing wells during thermal cycling | Transparent | 6/12 x 8-cap strip |

**Table A1 3. Reagents and materials provided in VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit with Ref. VS-VAR106L, VS-VAR106H, VS-VAR112L and VS-VAR112H.**

| Reagent/Material | Description | Color | Amount |
|---|---|---|---|
| *SARS-CoV-2 Variant I* 96-well plate | A mix of enzymes, primers probes, buffer, dNTPs, stabilizers in stabilized format | White | 1 plate |
| Rehydration Buffer | Solution to reconstitute the stabilized product | Blue | 1 vial x 1.8 mL |
| *SARS-CoV-2 Variant I* Positive Control | Non-infectious synthetic lyophilized cDNA | Red | 1 vial |
| Negative control | Non template control | Violet | 1 vial x 1 mL |
| Water RNAse/DNAse free | RNAse/DNAse free water | White | 1 vial x 1 mL |
| Tear-off 8-cap strips | Optical caps for sealing plate during thermal cycling | Transparent | 12 x 8-cap strip |

**Table A1 4. Reagents and materials provided in VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit with Ref VS-VAR113L and VS-VAR113H.**

## A1.3 Test procedure

### A1.3.1 Lyophilized positive control

*SARS-CoV-2 Variant I* Positive Control contains high copies of the template, the recommendation is to open and manipulate it in a separate laboratory area away from the other components. Reconstitute the lyophilized *SARS-CoV-2 Variant I* Positive Control (red vial) by adding 100 µL of the supplied Water RNAse/DNAse free (white vial) and vortex thoroughly.

Once the positive control has been re-suspended, store it at -20°C. It is recommended to separate it in aliquots to minimize freeze and thaw cycles.

### A1.3.2 PCR protocol

Determine and separate the number of required reactions including samples and controls. One positive and negative control must be included in each run for each assay. Peel off protective aluminium seal from plates or strips.

1) Reconstitute the number of wells you need.

Add 15 µL of Rehydration Buffer (blue vial) into each well.

2) Adding samples and controls.

Add 5 µL of RNA sample, reconstituted *SARS-CoV-2 Variant I* Positive Control (red vial) or Negative Control (violet vial) in different wells and close them with the provided caps. It is recommended to briefly centrifuge the 8-well strips or 96-well plate.

Load the plate or the strips in the thermocycler.

3) Set up the thermocycler (consult thermocycler compatibility on CerTest´s website (www.certest.es).

Program the thermocycler following the conditions listed below and start the run:

| Cycles | Step | Time | Temperature |
|---|---|---|---|
| **1** | Reverse transcription | 15 min | 45ºC |
| **1** | Initial denaturation | 2 min | 95ºC |
| **45** | Denaturation | 10 sec | 95ºC |
| | Annealing/Extension (Data collection*) | 50 sec | 63ºC |

**Table A1 5. PCR protocol**

Fluorogenic data should be collected during the extension step (*) through the FAM (E484K), HEX, JOE or VIC (K417N), ROX (K417T), and Cy5 (N501Y). Depending on the equipment used select the proper detection channel (to check most common detection channels consult website www.certest.es). In Applied Biosystems 7500 Fast Real-Time PCR System and Stratagene Mx3005P™ Real Time PCR System check that passive reference option ROX is none. In the Applied Biosystems 7500 Fast Real-Time PCR System select Ramp Speed Standard in Select New Experiment/Advanced Setup/Experiment Properties.

ANNEX 2

# TUBE FORMAT

Annex for the following references:

| PRODUCT | REFERENCE |
|---|---|
| VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit, 4 tubes x 24 reactions | VS-VAR196T |

Table A2. 1.References.

## A2.1 Principle of the procedure

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit contains in each Reaction-Mix tube all the components necessary for 24 real time PCR reactions (specific primers/probes, dNTPS, buffer, polymerase and retrotranscriptase) in a stabilized format.

| Target | Channel | Gene |
|---|---|---|
| E484K | FAM | *S* gene |
| K417N | HEX, VIC or JOE * | *S* gene |
| K417T | ROX | *S* gene |
| N501Y | Cy5 | *S* gene |

Table A2. 2.Target, channel and genes.

*Depending on the equipment used select the proper detection channel, channel, to check most common detection channels consult website www.certest.es.

## A2.2 Reagents provided

VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit includes the following materials and reagents detailed in Table A2.3.

| Reagent/Material | Description | Colour | Amount |
|---|---|---|---|
| *SARS-CoV-2 Variant I* Reaction-Mix tube | A mix of enzymes, primers probes, buffer, dNTPs, stabilizers in stabilized format | White | 4 vials |
| Rehydration Buffer | Solution to reconstitute the stabilized product | Blue | 1 vial x 1.8 mL |
| *SARS-CoV-2 Variant I* Positive Control | Non-infectious synthetic lyophilized cDNA | Red | 1 vial |
| Negative control | Non template control | Violet | 1 vial x 1 mL |
| Water RNAse/DNAse free | RNAse/DNAse free water | White | 1 vial x 1 mL |

Table A2. 3. Reagents and materials provided in VIASURE *SARS-CoV-2 Variant I* Real Time PCR Detection Kit with Ref. VS-VAR196T.

## A2.3 Test procedure

### A2.3.1 Lyophilized positive control

*SARS-CoV-2 Variant I* Positive Control contains high copies of the template, the recommendation is to open and manipulate it in a separate laboratory area away from the other components. Reconstitute the lyophilized *SARS-*

*CoV-2 Variant I* Positive Control (red vial) by adding 100 µL of the supplied Water RNAse/DNAse free (white vial) and vortex thoroughly.

Once the positive control has been re-suspended, store it at -20ºC. It is recommended to separate it in aliquots to minimize freeze and thaw cycles.

### A2.3.2 Lyophilized reaction mix tube

Determine the number of required reactions including samples and controls (one positive and negative control must be included in each run). Obtain the correct number of lyophilized Reaction-Mix vials (24-reactions each one) for testing.

Recommendation is to open and manipulate the *SARS-CoV-2 Variant I* Reaction-Mix tube in pre-PCR laboratory area. Open lyophilized Reaction-mix tube (white vial) carefully to avoid disruption of the pellet and add 390 µL of Rehydration Buffer (blue vial) supplied. Mix gently by pipetting up and down. Spin down briefly to remove bubbles generated during mixing.

Once the Reaction-Mix tube has been re-suspended, return unused reagents to the appropriate storage conditions at -20ºC. Recommendation is to separate it in aliquots to minimize freeze and thaw cycles.

Note: The volume of the rehydrated Reaction-Mix is sufficient for 24 reactions. The rehydrated Reaction-Mix may be kept at 25ºC±5ºC or 2-8ºC for up to 4-hours (see Transport and storage conditions section for additional storage options).

### A2.3.3 PCR protocol

1) Adding rehydrated Reaction-Mix to the number of required wells.

Add 15 µL of rehydrated *SARS-CoV-2 Variant I* Reaction-Mix (white vial) into each tube.

2) Adding samples and controls.

Add 5 µL of RNA sample, reconstituted *SARS-CoV-2 Variant I* Positive Control (red vial) or Negative Control (violet vial) in different wells and close the tubes with caps or seal the plate. Centrifuge briefly.

Load the plate, the strips, or tube in the thermocycler.

3) Set up the thermocycler (consult thermocycler compatibility on CerTest´s website www.certest.es).

Program the thermocycler following the conditions listed below and start the run:

| Cycles | Step | Time | Temperature |
|---|---|---|---|
| **1** | Reverse transcription | 15 min | 45ºC |
| **1** | Initial denaturation | 2 min | 95ºC |
| **45** | Denaturation | 10 sec | 95ºC |
| | Annealing/Extension (Data collection*) | 50 sec | 63ºC |

Table A2. 4. PCR protocol.

Fluorogenic data should be collected during the extension step (*) through the FAM (E484K), HEX, JOE or VIC (K417N), ROX (K417T), and Cy5 (N501Y). Depending on the equipment used select the proper detection channel (to check most common detection channels consult website www.certest.es). In Applied Biosystems 7500 Fast Real-

Time PCR System and Stratagene Mx3005P™ Real Time PCR System check that passive reference option ROX is none. In the Applied Biosystems 7500 Fast Real-Time PCR System select Ramp Speed Standard in Select New Experiment/Advanced Setup/Experiment Properties.

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## Symbols for IVD components and reagents/Símbolos para reactivos y productos para diagnóstico *in vitro*

| | | | |
|---|---|---|---|
| IVD — *In vitro* diagnostic device / Producto para diagnóstico *in vitro* | Keep dry / Almacenar en lugar seco | Use by / Fecha de caducidad | Manufacturer / Fabricante |
| LOT — Batch code / Número de lote | Consult instructions for use / Consultar las instrucciones de uso | Temperature limitation / Limitación de temperatura | Contains sufficient for <n> test / Contiene <n> test |
| UDI — Unique Device Identification / Identificación única de dispositivo | REF — Catalogue number / Número de referencia | | |

## Trademarks



| Control de Cambios / Change Control | | |
|---|---|---|
| **Versión / Version nº** | **Cambios / Changes** | **Fecha / Date** |
| 00 | Versión Original / Original Version | 25/05/2021 |
| | | |
| | | |

**Table A 3. Tabla de Control de Cambios / Control change table.**

Revision: 25th May 2021

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