VIASURE

Real Time PCR Detection Kit

SARS-CoV-2 Variant

for BD MAX™ System

CE IVD

These instructions for use apply to the following reference / *Denne brugsanvisning gælder for følgende reference:*

PRODUCT / PRODUKT	REFERENCE / REFERENCE
VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System	444216 / VS-USB124

Table A 1. Reference for product to be used with the BD MAX™ System. / *Reference til produkt, der skal bruges med BD MAX™ System.*

Content

Indholdsfortegnelse

ENGLISH

1. Intended use

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System is an automated real-time RT-PCR test designed for the qualitative detection of HV 69/70 deletion, K417N mutation and K417T mutation in the *S* gene of SARS-CoV-2, associated to SARS-CoV-2 Alpha (lineage B.1.1.7), Beta (lineage B.1.351) and Gamma (linage P.1) variants, in nasopharyngeal and oropharyngeal swabs and saliva samples.

The assay is intended to be used with SARS-CoV-2 positive samples or, when the test is performed in conjunction with the VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215) with samples from patients suspected Coronavirus disease 2019 (COVID-19) by their healthcare professional (HCP).

This test is intended to be used as an aid to monitor the prevalence of variants that carry the HV 69/70 deletion, K417N or K417T mutations in the *S* gene and to assist in control measures. The assay uses the BD MAX™ System for automated extraction of RNA and subsequent real-time RT-PCR, employing the reagents provided combined with universal reagents and disposables for the BD MAX™ System. RNA is extracted from specimens, and complementary DNA (cDNA) is synthetized and amplified using RT-PCR and detected using fluorescent reporter dye probes specific for HV 69/70 deletion, K417N or K417T mutations.

2. Summary and Explanation

Coronavirus are enveloped non-segmented positive-sense RNA viruses and belong to *Coronaviridae* family [1,2]. There are six coronavirus species known to cause human diseases [2]. Four viruses (229E, OC43, NL63 and HKU1) cause common cold symptoms and the other two (severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)) are zoonotic and produce more severe complications [2]. SARS-CoV and MERS-CoV have caused more than 10,000 cumulative cases in the past two decades, with mortality rates of 34% MERS-CoV and 10% SARS-CoV [1,3].

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 [2,4]. 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 [5].

Human-to-human transmission of the SARS-CoV-2 has been confirmed, even in the incubation period without symptoms, and the virus causes severe respiratory illness like those SARS-CoV produced [1,6,7,8]. 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 [1,4]. 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, fatigue, anorexia, myalgia and dyspnea [1,4,6,9]. Less common symptoms are sore throat, nasal congestion, headache, diarrhea, nausea and vomiting [1,4]. Loss of smell (anosmia) or loss of taste (ageusia) preceding the onset of respiratory symptoms has also been reported [9]. Older adults and people who have severe underlying medical conditions like heart or lung disease or diabetes seem to be at higher risk for developing more serious complications from COVID-19 illness [10].

Diagnosis of COVID-19 is performed detecting conventional causes of pneumonia early and detected by next-generation sequencing or real-time RT-PCR methods [1,11]. Several assays that detect the SARS-CoV-2 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) [12].

CDC recommends upper respiratory tract specimens (nasopharyngeal (NP) and oropharyngeal (OP) swabs, nasal mid-turbinate swab, nasal swab, nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) and saliva specimens collected mainly by a healthcare professional) 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 [11]. In addition, other clinical specimens as blood, urine and stool may be collected to monitor the presence of the virus [11,12].

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 Alpha variant (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 presence of the HV 69/70 deletion is associated with the Alpha 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.

The Beta (B.1.351) variant was 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 Gamma) 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 is

considered a Variant Under Monitoring (VUM) and only harbors the mutation E484K. The P.2 lineage has been detected as the most prevalent variant in several Amazonas 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. For this reason, VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System allows the detection of HV 69/70 deletion, K417N or K417T mutations associated with Variants of Concern Alpha, Beta and Gamma.

3. Principle of the procedure

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System is designed for the qualitative detection of RNA with HV 69/70 deletion, K417N mutation and K417T mutation in the *S* gene of SARS-CoV-2 from nasopharyngeal and oropharyngeal swabs and saliva samples. The detection is done in one step real-time RT-PCR format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction tube. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase, which is followed by the amplification of a conserved region of *S* gene for SARS-CoV-2 for HV 69/70 deletion, K417N mutation and K417T mutation using specific primers and fluorescent-labeled probes.

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System is based on 5´ exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bound 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 the target template. This fluorescence is measured on the BD MAX™ System.

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System contains in each tube all the components necessary for real-time PCR assay (specific primers/probes, dNTPs, buffer, polymerase, reverse-transcriptase) in a stabilized format, as well as an endogenous internal control to monitor the extraction process and/or inhibition of the polymerase activity. The assay uses a human housekeeping gene as an Endogenous Internal Control (IC) (human *RNase P* gene). Human housekeeping genes are involved in basic cell maintenance and, therefore, are expected to be present in all nucleated human cells and maintain relatively constant expression levels.

Target	Channel	Gene
HV 69/70 deletion	475/520	*S* gene
K417N mutation	530/565	*S* gene
K417T mutation	585/630	*S* gene
Endogenous Internal Control (IC)	630/665	human *RNase P* gene

Table 1. Target, channel and genes.

4. Reagents provided

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System includes the following materials and reagents detailed in Table 2:

Reagent/Material	Description	Color or Barcode	Amount
SARS-CoV-2 Variant reaction tube	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and endogenous internal control in stabilized format	Green foil	2 pouches of 12 transparent tubes
Rehydration Buffer tube	Solution to reconstitute the stabilized product	11 foil	1 pouch of 24 transparent tubes

Table 2. Reagents and materials provided in VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System with Cat. N°. VS-USB124 (444216).

5. Reagents and equipment to be supplied by the user

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

- Real-time PCR instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442827 or 442828).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).
- Filter tips.
- Powder-free disposable gloves.
- Optional: VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215)

6. Transport and storage conditions

- The kits can be shipped and stored at 2-40ºC until the expiration date which is stated on the label.
- After opening the aluminum pouches which contain the reaction tubes, the product can be used up to 28 days.

7. Precautions for users

- The product is intended for use by professional users only, such as laboratory or health care professionals and technicians, trained in molecular biological techniques.
- 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.

- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- 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.
- Protect reagents from humidity. Prolonged exposure to humidity may affect product performance.
- Keep components away from light.
- 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* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, any additional reagents required for testing and the BD MAX™ System are not contaminated. Always avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents. 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 and cartridges (BD MAX™ PCR Cartridge).
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridge are designed to prevent contamination.
- 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.
- 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.
- Samples 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.
- If the kit is used in combination with the VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215), please refer to the corresponding instructions for use.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Test procedure

8.1. Sample collection, storage and transport

The VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System has been tested on nasopharyngeal swabs and saliva samples, both collected in viral transport medium (VTM) – Vircell S.L. -; BD™ Universal Viral Transport (UVT) System media – BD - or IMPROVIRAL™ Viral Preservative Medium (VPM) -Guangzhou Improve Medical Instruments Co. Ltd and oropharyngeal swabs collected in viral transport medium (VTM) - Vircell. Other types of samples must be validated by the user.

Collection, storage and transport of specimens should be maintained per the conditions validated by the user. Overall, respiratory and saliva 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), we recommend 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 nasopharyngeal/oropharyngeal swabs and saliva specimens must be collected, transported and stored 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. Sample preparation and RNA extraction

Perform the sample preparation according to the recommendations in the instructions for use of extraction kit used, BD MAX™ ExK™ TNA-3. Note that some other samples may require pre-processing. Application-specific extraction preparation procedures should be developed and validated by the user.

When using nasopharyngeal or oropharyngeal specimens:

1. Pipette between 400 and 750 µL of nasopharyngeal or oropharyngeal swab collected in viral transport media (VTM) or in BD™ Universal Viral Transport (UVT) System media into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

In case of using saliva samples collected in transport media:

1. Saliva samples may be collected in Viral Transport Medium (VTM), BD™ Universal Viral Transport (UVT), or IMPROVIRAL™ Viral Preservative Medium (VPM) at a ratio of 1:3 (saliva:media). Vortex for 1 minute at high speed. Pipette 750 µL into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap.

Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

In case of using neat saliva samples:

1. Combine saliva with Viral Transport Medium (VTM), BD™ Universal Viral Transport (UVT), or IMPROVIRAL™ Viral Preservative Medium (VPM) so that the final ratio of saliva:media is 1:3. Vortex for 1 minute at high speed. Then pipette 750 µL into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

8.3. PCR protocol

Note: Please, refer to the BD MAX™ System User's Manual for detailed instructions.

8.3.1. Creating PCR test program for VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System

Note: If you have already created the test for the VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System, you can skip step 8.3.1 and go directly to 8.3.2.

1) On the "Run" screen of the BD MAX™ System, select the "Test Editor" tab.
2) Click the "Create" button.
3) In the Basic Information tab, within the "Test Name" window, name your test: i.e. VIASURE *SARS-CoV-2 Variant*.
4) In the "Extraction Type" drop down menu, select "ExK TNA-3".
5) In the "Master Mix Format" drop down menu, choose "Type 5".
    a. Note: Product may be used in combination with the VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215), then select "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)".
6) In the "Sample extraction parameters" select "User defined" and adjust sample volume to 950 µL.
7) In the "Ct Calculation" select "Call Ct at Threshold Crossing".
8) If running software version 5.00 or higher, in the "Custom Barcodes" select the following configuration:
    a. Snap-In 2 Barcode: leave empty (concerning *SARS-CoV-2 Variant* reaction tube no barcode configuration is needed).
    b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube).
    c. Snap-In 4 Barcode: 1G if used in combination with *SARS-CoV-2 (N1 + N2)* reaction tube and the format "Dual Master mix Concentrated Lyophilized MM with rehydration Buffer (Type 5)" (Section 8.3.1).
9) In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 3).

a. Note: Product may be used in combination with the VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215), "PCR Settings" and "Test Steps" should be completed for Snap-In 4 (blue) position (see the corresponding instructions for use).

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	HV69-70	80	150	0	40
530/565 (HEX)	K417N	80	150	0	40
585/630 (ROX)	K417T	80	150	0	40
630/665 (Cy5)	IC	80	150	0	35
680/715 (Cy5.5)	-	0	0	0	0

Table 3. PCR settings.

Note: It is recommended to set the minimum threshold values listed above for each channel as a starting point, but the final settings must be determined by the end-user during the result interpretation, 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.

10) In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 4), as well.

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	3.0	0.0	0.0	0.0
	530/565	1.0	-	0.0	0.0	0.0
	585/630	0.0	0.0	-	0.0	0.0
	630/665	0.0	0.0	5.0	-	0.0
	680/715	0.0	0.0	0.0	0.0	-

Table 4. Spectral cross-talk parameters.

11) In "Test Steps" tab, enter the PCR protocol (Table 5).

Step Name	Profile Type	Cycles	Time (s)	Temperature	Detect
Reverse transcription	Hold	1	900	45°C	-
Initial denaturation	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection)	2-Temperature	45	10	95°C	-
			61.1	63°C	✓

Table 5. PCR protocol.

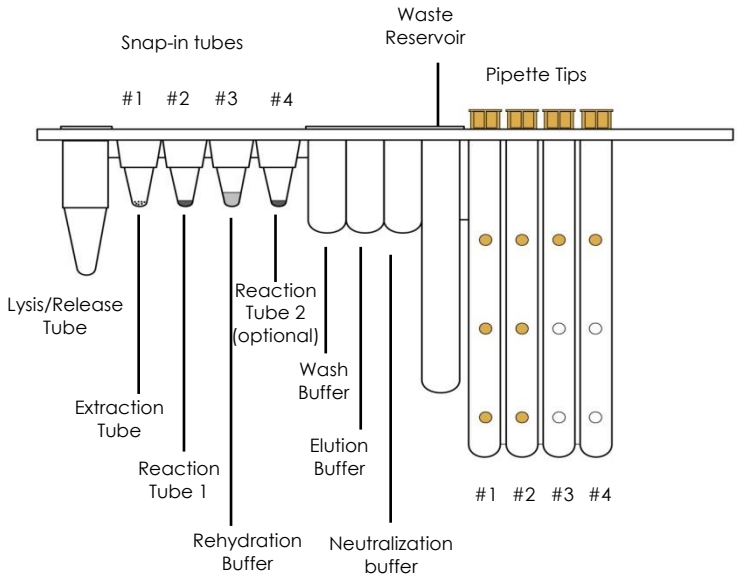
12) Click the "Save Test" button.

8.3.2. BD MAX™ Rack set up

1) For each sample to be tested, remove one Unitized Reagent Strips from the BD MAX™ ExK™ TNA-3 kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes and load on the BD MAX™ System sample racks.
2) Remove the required number of BD MAX™ ExK™ TNA Extraction Tubes (B4) (white foil) from their protective pouch. Snap the Extraction Tube(s) (white foil) into its corresponding positions in the TNA strip (Snap position 1, white color coding on the rack. See Figure 1). Remove excess air, and close pouch with the zip seal.

3) Determine and separate the appropriate number of *SARS-CoV-2 Variant* reaction tubes (green foil) and snap into their corresponding positions in the strip (Snap position 2, green color coding on the rack. See Figure 1).
    a. Remove excess air, and close aluminum pouches with the zip seal.
    b. In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
        i. Note: If you choose the format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Section 8.3.1), determine and separate the appropriate number of additional *SARS-CoV-2* reaction tubes (1G foil in case of VIASURE SARS-CoV-2 (N1+N2) test) and snap into their corresponding positions in the strip (Snap position 4, blue color coding on the rack. See Figure 1). Remove excess air, and close aluminum pouches with the zip seal.
4) Remove the required number of Rehydration Buffer tubes (11 foil) and snap into their corresponding positions in the strip (Snap position 3, non-color coding on the rack. See Figure 1). Remove excess air and close the pouch with the zip seal.
    a. In order to ensure a correct transfer, please make sure that the liquid is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the buffer is at the bottom of the tube.

Figure 1. BD MAX™ TNA Reagent Strip (TNA) from the BD MAX™ ExK™ TNA-3 kit.



8.3.3. BD MAX™ Instrument set up

1) Select the "Work List" tab on the "Run" screen of the BD MAX™ System software v4.50A or higher.
2) In the "Test" drop down menu, select VIASURE *SARS-CoV-2 Variant* (if not already created see Section 8.3.1).
3) Select the appropriate kit lot number (found on the outer box of extraction kit used) from the pull-down menu (optional).

4) Enter the Sample Buffer Tube identification number into the Sample tube window of the Work List, either by scanning the barcode with the scanner or by manual entry.
5) Fill the Specimen/Patient ID and/or Accession window of the Work List and click the "Save" button. Continue until all Sample Buffer Tubes are entered. Ensure that the specimen/patient ID and the Sample Buffer Tubes are accurately matched.
6) Place the prepared Sample Buffer Tube into the BD MAX™ Rack(s).
7) Load the rack(s) into the BD MAX™ System (Rack A is positioned on the left side of the BD MAX™ System and Rack B on the right side).
8) Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System.
9) Close the BD MAX™ System door.
10) Click "Start Run" to begin the procedure.

8.3.4. BD MAX™ report

1) In main menu, click the "Results" button.
2) Either double click on your run in the list or press the "view button".
3) Click on "Print", select: "Run Details, Test Details and Plot…".
4) Click on "Print or Export button" on the "Run Reports" screen.

9. Result interpretation

For a detailed description on how to analyze data, refer to the BD MAX™ System User's manual.

Analysis of the VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System is intended to be performed as a reflex on samples with positive result for SARS-CoV-2 RNA. If used in conjunction with VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System on samples of unknown status for presence of SARS-CoV-2 RNA, please refer to those instructions for use for results interpretation for determination of the SARS-CoV-2 RNA result.

The analysis of the data is done by the BD MAX™ software according to the manufacturer´s instructions. The BD MAX™ software reports Ct values and amplification curves for each detector channel of each sample tested in the following way:

- Ct value of 0 indicates that there was no Ct value calculated by the software with the specified Threshold (see Table 3). Amplification curve of the sample showing a "0" Ct value must be checked manually.

- Ct value of -1 indicates that no amplification process has occurred.

- Any other Ct value should be interpreted in correlation with the amplification curve and according to the sample interpretation guidelines outlined in Table 6.

Check Internal Control signal to verify the correct functioning of the amplification mix. In addition, check that there is no report of BD MAX™ System failure.

Results should be read and analyzed using the following table:

HV 69/70 deletion target (475/520)	K417N mutation target (530/565)	K417T mutation target (585/630)	Endogenous Internal Control (630/665)	Interpretation
+	-	-	+/-[1]	**HV 69/70 deletion Detected [1]**
-	+	-	+/-[1]	**K417N mutation Detected [1]**
-	-	+	+/-[1]	**K417T mutation Detected [1]**
+	+	-	+/-[1]	**HV 69/70 deletion and K417N mutation Detected [1]**
+	-	+	+/-[1]	**HV 69/70 deletion and K417T mutation Detected [1]**
-	+	+	+/-[1]	**K417N and K417T mutation Detected [1]**
+	+	+	+/-[1]	**HV 69/70 deletion, K417N mutation and K417T mutation Detected [1]**
-	-	-	+[1]	**HV 69/70 deletion, K417N mutation and K417T mutation not Detected [1]**
-	-	-	-[2]	**Unresolved (UNR) Result obtained in the presence of inhibitors in the PCR reaction or when a general problem (not reported by an error code) with the sample processing and/or amplification steps occurs.[2]**
IND	IND	IND	IND	**Indeterminate assay result (IND). Due to BD MAX™ System failure. Assay result displayed in case of an instrument failure linked to an error code.**
INC	INC	INC	INC	**Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run.**

Table 6. Sample interpretation.

+: Amplification occurred.

-: No amplification occurred.

1 A sample is considered positive if the Ct value obtained is less than 40. The endogenous Internal Control (IC) may or may not show an amplification signal. Sometimes, the IC detection is not necessary because a high copy number of the target can cause preferential amplification of target-specific nucleic acids.

2 In the case of HV 69/70 deletion, K417N mutation and K417T mutation targets sites negative, IC must show an amplification signal with Ct less than 35. The Ct value could be very variable due to the Endogenous Internal Control is a human housekeeping gene that should be present in all human nucleated cells in the original sample. If there is an absence of signal or Ct value ≥ 35 of the Endogenous Internal Control, the result is considered as 'Unresolved', and retesting is required.

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

Lineages	WHO label	Mutations in the S gene [1]		
		HV 69/70 deletion	K417N mutation	K417T mutation
B.1.1.7	Alpha	X	-	-
B.1.351	Beta	-	X	-
P.1	Gamma	-	-	X

Table 7. Summary of mutations associated with known Variants of Concern (VOC).

[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 HV 69/70 deletion and mutations K417T and K417N 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.

The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

10. Limitations of the test

- The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.
- Although this assay can be used with other types of samples it has been validated with nasopharyngeal/oropharyngeal swabs and saliva samples, all collected in Viral Transport Medium (VTM).
- For good test performance, the lyophilized product should be at the bottom of the tube and not adhered to the top area of the tube or the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
- 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.
- The quality of the test depends on the quality of the sample; proper extracted nucleic acid from respiratory 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 with HV 69/70 deletion, K417N mutation or K417T mutation in the *S* gene, either samples containing high concentrations of target RNA or contamination due to PCR products from previous reactions.
- The specific primer and probe combinations for detection of HV 69/70 deletion, K417N mutation or K417T mutation used in VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System do not show significant combined homologies with the human genome, human microflora, or other coronaviruses, which might result in predictable false positive.
- 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 variant.
- 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.

- Some samples may fail to exhibit *RNase P* amplification curves due to low human cell numbers in the original clinical sample. A negative IC signal does not preclude the presence of HV 69/70 deletion, K417N mutation or K417T mutation in a clinical specimen.
- 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 HV 69/70 deletion is associated with the Alpha variant (lineage B.1.1.7), K417N mutation with Beta variant (lineage B.1.351) and K417T mutation with Gamma variant (lineage P.1), 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 be used with positive SARS-CoV-2 samples.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System, retesting will be required. Unresolved results may be due to the presence of inhibitors in the sample or an incorrect rehydration of lyophilized reaction mix tube. If there is an instrument failure, Indeterminate or Incomplete results will be obtained.

11. Quality control

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System contains an Endogenous Internal Control (IC) in each reaction tube which confirms the correct performance of the technique.

12. Performance characteristics

12.1. Clinical sensitivity and specificity

The clinical performance of VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System was tested using respiratory clinical samples (nasopharyngeal swabs) from patients with suspected respiratory infection. The results were as follows:

	Site	Sample type	Workflow	Target
1	CerTest Biotec S.L (Zaragoza, Spain)	nasopharyngeal swab	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	HV 69/70 deletion
				Mutation K417T
				Mutation K417N

Table 8. Site, sample type, workflow and target.

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

Site	Comparator assay	Target	TP	TN	FP	FN	Sensitivity	Specificity
1	TaqPath COVID-19 CE-IVD RT-PCR Kit/ VIASURE *SARS-CoV-2* Real Time PCR Detection Kit molecular assay + sequencing	HV 69/70 deletion	48	167	0	2	96% (85 – 99)	100% (97 – 100)
		Mutation K417T	50	167	0	0	100% (91 – 100)	100% (97 – 100)
		Mutation K417N	7	209	0	1	88% (46 – 99)	100% (97 – 100)

Table 9. True positive and negative values, false positive and negative values, sensitivity, specificity for VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System.

Result show agreement to detect the HV 69/70 deletion, K417T and K417N SARS-CoV-2 mutations using VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System.

In order to evaluate the compatibility of different sample matrices (nasopharyngeal swab, oropharyngeal swab and nasopharyngeal/oropharyngeal swab in Viral Transport Medium (VTM) from Vircell), a compatibility study have been carried out. The obtained results showed that the three different sample matrices were compatible with the VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytical sensitivity

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System detection limit (LoD) results with a positive rate of ≥ 95% are as follows:

a) VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of ≥ 2 genome copies/reaction on nasopharyngeal samples and ≥ 5 genome copies/reaction on saliva samples for HV 69/70 deletion measured using the SARS-CoV-2 B.1.1.7 lineage.

b) VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of ≥ 5 genome copies/reaction on nasopharyngeal samples and ≥ 5 genome copies/reaction on saliva samples for K417N mutation measured using the SARS-CoV-2 B.1.351 lineage.

c) VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of ≥ 10 genome copies/reaction on nasopharyngeal samples and ≥ 15 genome copies/reaction on saliva samples for K417T mutation measured using the SARS-CoV-2 P.1 lineage.

Figure 2. Dilution series of SARS-CoV-2 Variant (HV 69/70 deletion) (synthetic cDNA) ($5.3*10^5$ -$5.2*10^1$ genome copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).

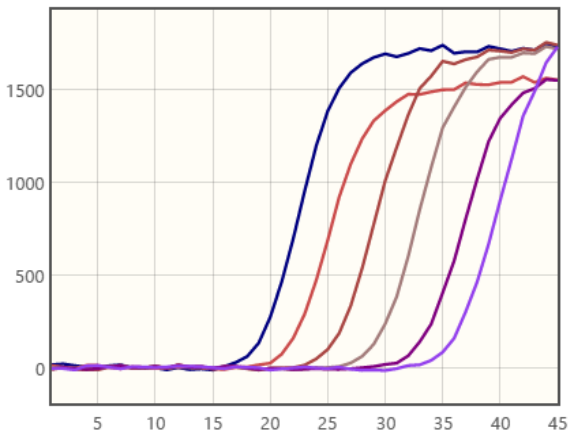


Figure 3. Dilution series of SARS-CoV-2 Variant (K417N mutation) (synthetic cDNA) ($5.3*10^5$ -$5.2*10^1$ genome copies per reaction) template run on the BD MAX™ System (530/565 (HEX) channel).

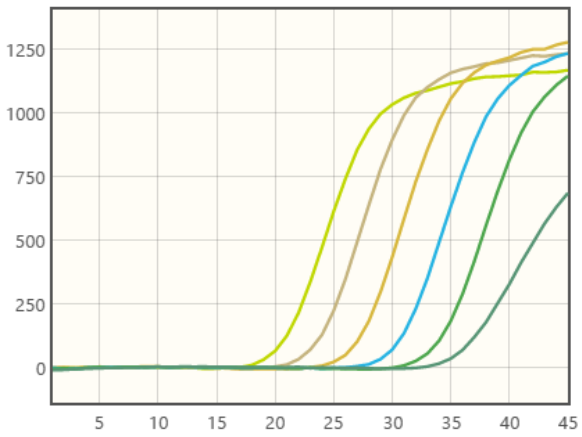
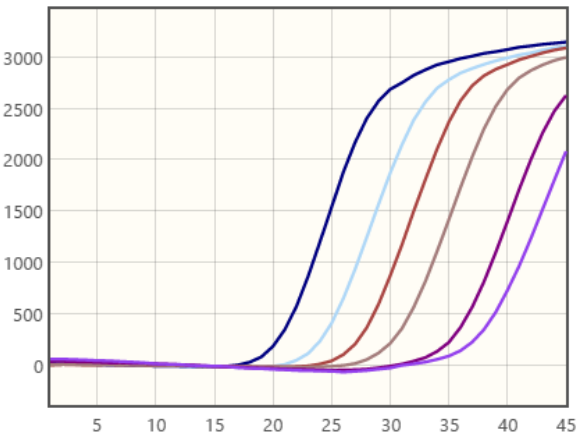


Figure 4. Dilution series of SARS-CoV-2 Variant (K417T mutation) (synthetic cDNA) ($5.3*10^5$ -$5.2*10^1$ genome copies per reaction) template run on the BD MAX™ System (585/630 (ROX) channel).



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:

Cross-reactivity testing					
Human Adenovirus types 1-5, 8, 15, 31, 40 and 41	-	Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-	Mycoplasma pneumoniae	-
Bocavirus	-	Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-	Mycobacterium tuberculosis	-
Bordetella bronchiseptica	-	Influenza A/Thüringen/5/17 (H3N2) virus	-	Human parainfluenza 1, 2, 3 and 4 viruses	-
Bordetella holmesii	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-	Pneumocytis jirovecii Type A1 and g885652	-
Bordetella parapertussis	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-	Human rhinovirus	-
Bordetella pertussis	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus	-	Respiratory syncytial virus (RSV) A/B	-
Chlamydia caviae	-	Influenza A/Anhui/1/2013 (H7N9) virus	-	SARS Coronavirus Strain Frankfurt 1	-
Chlamydia psittaci genotype A and C	-	Influenza B/Brisbane/60/2008 virus	-	Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1*	-
Chlamydophila pneumoniae CM-1	-	Influenza A/South Australia/55/2014, IVR-175	-	Human 2019-nCoV strain 2019-nCoV/Italy-INMI1*	-
Human coronavirus 229E, OC43, NL63 and HKU1	-	Influenza B/Phuket/3073/2013 virus	-	MT007544.1(SARS-CoV-2 isolate Australia/VIC01/2020)*	-
MERS Coronavirus	-	Influenza B/Florida/04/06 virus	-	MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1)*	-
Enterovirus Coxsackievirus A24, A9 and B3	-	Legionella bozemanii	-	SARS-CoV-2 strain 2019nCoV/USAWA1/2020*	-
Enterovirus Echovirus 30	-	Legionella dumoffii	-	SARS-CoV-2 BetaCoV/Berlin/ChVir1670/2020_IsolatBER*	-
Enterovirus 68, 71	-	Legionella longbeachae	-	SARS-CoV-2 BetaCoV/Munich/ChVir984/2020*	-
Haemophilus influenzae MinnA	-	Legionella micdadei	-	SARS-CoV-2 BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER*	-
Influenza A/New Caledonia/20/99(H1N1) virus	-	Legionella pneumophila	-	Staphylococcus aureus	-
Influenza A/Victoria/210/2009 (H3N2)	-	Human metapneumovirus A and B	-	Streptococcus pneumoniae	-
Influenza A/California/7/2009(H1N1) pdm09 virus	-	Moraxella catarrhalis	-	Streptococcus pyogenes	-

Table 10. 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 HV 69/70 deletion, K417N mutation and K417T mutation in the *S* gene present in SARS-CoV-2 Alpha, Beta and Gamma variants (lineages B.1.1.7, B.1.351 and P.1), among others.

12.4. Analytical reactivity

The reactivity of VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System was evaluated against synthetic RNA controls for two different sequences associated to the Alpha variant (B.1.1.7_710528 UK Variant and B.1.1.7_601443 UK Variant), one sequence associated to the Beta Variant (Control 16, SARS-CoV-2 lineage B.1.351 South Africa/KRISP-ECK005299/2020) and one sequence associated to the Gamma variant (Control 17, SARS-CoV-2 lineage P.1 Japan/Brasilian variant Japan/IC-0564/2021), showing positive results.

DANSK

1. Anvendelsesformål

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System er en automatiseret RT-PCR-test i realtid designet til den kvalitative påvisning af HV 69/70-deletion, K417N-mutation og K417T-mutation i *S*-genet i SARS-CoV-2, associeret med SARS-CoV-2 Alpha (B. 1.1.7 stamme), Beta (B.1.351 stamme) og Gamma (P.1-stamme) varianter i podninger fra næsesvælgrum, svælg (oropharynx) og spytprøver.

Analysen er beregnet til at blive brugt med SARS-CoV-2 positive prøver eller, når testen udføres i forbindelse med VIASURE *SARS-CoV-2 (N1 + N2)* Realtids PCR-detektionssæt til BD MAX ™ -system (Ref: 444215) med prøver fra patienter, der har mistanke om Coronavirus-sygdom 2019 (COVID-19) af deres sundhedspersonale (HCP).

Denne test er beregnet til at blive brugt som et hjælpemiddel til at overvåge forekomsten af varianter, der bærer HV 69/70-deletions-, K417N- eller K417T-mutationer i *S*-genet og til at hjælpe med kontrolforanstaltninger. Analysen anvender BD MAX™ System til automatisk ekstraktion af RNA og efterfølgende realtids-RT-PCR med anvendelse af de medfølgende reagenser kombineret med universelle reagenser og engangsartikler til BD MAX™ System. RNA ekstraheres fra prøver, og komplementært DNA (cDNA) syntetiseres og amplificeres ved hjælp af RT-PCR og detekteres ved hjælp af fluorescerende reporterfarvestoffer, der er specifikke for HV 69/70 deletion, K417N eller K417T mutationer.

2. Oversigt og forklaring

Coronavirus er omsluttet ikke-segmenterede RNA-vira med positiv sans og tilhører *Coronaviridae*-familien [1,2]. Seks coronavirusarter vides at forårsage sygdomme hos mennesker [2]. Fire vira (229E, OC43, NL63 og HKU1) forårsager almindelige forkølelsessymptomer, og de to andre (svært akut respiratorisk syndrom coronavirus (SARS-CoV) og Mellemøstens respiratoriske syndrom coronavirus (MERS-CoV)) er zoonotiske og forårsager mere alvorlige komplikationer [2]. SARS-CoV og MERS-CoV har forårsaget mere end 10.000 kumulative tilfælde i de seneste to årtier med en dødelighed på 34 % MERS-CoV og 10 % SARS-CoV [1,3].

I december 2019 havde nogle mennesker, der arbejdede på eller boede omkring Huanan skaldyrsmarked i Wuhan, Hubei-provinsen i Kina, lungebetændelse af ukendt årsag [2,4]. Dyb sekvensanalyse af respirationsprøverne indikerede en ny coronavirus, som først fik navnet 2019 novel coronavirus (2019-nCoV) og for nylig SARS-CoV-2 [5].

Overførsel af SARS-CoV-2 fra menneske til menneske er blevet bekræftet, selv i inkubationsperioden uden symptomer, og virusset forårsager alvorlige luftvejslidelser, som ligner dem SARS-CoV frembragte [1,6,7,8]. Selv om lungebetændelse er den hyppigste sygdom, har enkelte patienter udviklet svær lungebetændelse, lungeødem, akut åndedrætsbesvær eller multiorgansvigt og død [1,4]. Centers of Disease Control and Prevention (CDC) mener, at symptomer på SARS-CoV-2 kan opstå i løbet af så få som 2 dage eller så længe som 14 dage efter eksponering, hvor de mest almindelige symptomer er feber, elle kulderystelser, hoste, træthed, anoreksi, myalgi og dyspnø [1,4,6,9]. Mindre almindelige symptomer er ondt i halsen, næsestop, hovedpine, diarré, kvalme og opkastning [1,4]. Tab af lugt (anosmi) eller tab af smag (ageusi) forud for forekomsten af luftvejssymptomer er også blevet rapporteret [9]. Ældre voksne og personer, der har alvorlige underliggende medicinske tilstande som hjerte- eller

lungesygdom eller diabetes, synes at have en højere risiko for at udvikle mere alvorlige komplikationer som følge af COVID-19-sygdom [10].

Diagnosticering af COVID-19 udføres ved tidlig påvisning af konventionelle årsager til lungebetændelsen og påvises ved next-generation-sekventering- eller realtids-RT-PCR-metoder [1,11]. Flere analyser, der påviser SARS-CoV-2 er tilgængelige i øjeblikket, såsom China CDC (genmål, *ORF1ab* og *N*), *Charité* – Germany (genmål, *RdRP* og *E*) eller US CDC (til mål i *N*-gen) [12].

CDC anbefaler prøver fra de øvre luftveje (nasofaryngeale (NP) og orofaryngeale (OP) podepindsprøver, nasal midt-turbinat-podepind, nasal podepind, nasofaryngeal vask/aspirat eller nasal vask/aspirat (NW) prøver indsamlet hovedsagelig af en sundhedsperson) og/eller prøver fra de nedre luftveje (sputum, endotrakealt aspirat eller bronkoalveolær skylning hos patienter med mere alvorlig luftvejssygdom) til identifikation af SARS-CoV-2 [11]. Derudover kan der udtages andre kliniske prøver såsom blod, urin og afføring for at overvåge tilstedeværelsen af virusset [11,12].

Siden den oprindelige genomiske karakterisering af SARS-CoV-2 er virussen blevet opdelt i forskellige genetiske grupper eller klynger (S, L, V, G med GH- og GR-undergrupper). Udseendet af mutationer er en naturlig og forventet begivenhed inden for udviklingsprocessen for virussen. Faktisk definerer nogle specifikke mutationer de virale genetiske grupper, der i øjeblikket cirkulerer globalt. De hidtil identificerede mutationer forbliver inden for de forventede mønstre for en coronavirus. Virus, der er klassificeret i den genetiske gruppe G, er de hyppigste i verden. Takket være den genetiske sekventering af patogenet rundt om i verden var det muligt at etablere dispersionsmønstre og virusudvikling.

Den 14. december 2020 erklærede Det Forenede Kongerige en stigning i forekomsten af SARS-CoV-2 i nogle regioner i sit land forbundet med en ny variant af virussen med angiveligt større transmissionskapacitet. Denne variant, kaldet Alpha-variant (B.1.1.7), præsenterede 23 forskellige mutationer: 13 ikke-synonyme, herunder en række mutationer i spike-proteinet (S), 4 deletioner og 6 synonyme. Ved udgangen af december var denne variant blevet opdaget i 31 lande og territorier i 5 af de 6 WHO-regioner. En af mutationerne er sletningen i positionerne 69-70 i spidsproteinet. Påvisning af HV 69/70-sletningen er af stor betydning, da det har været relateret til immunlækage hos immunsupprimerede patienter og til øget virusinfektivitet. En anden grund til bekymring i relation til HV 69/70-sletningen er, at den påvirker følsomheden af virusdetektion ved hjælp af molekylære teknikker (RT-PCR), der detekterer S-genet.

Tilstedeværelsen af HV 69/70 -sletningen er forbundet med Alpha -varianten, afstamning B.1.1.7, men andre varianter såsom B.1.1.298 (dansk slægt) eller B.1.258 har også denne sletning.

Beta (B.1.351) -varianten blev først identificeret i Nelson Mandela Bay, Sydafrika, i prøver, der går tilbage til begyndelsen af oktober 2020. Varianten blev også identificeret i Zambia i slutningen af december 2020, hvor det så ud til at være den dominerende variant i landet. Denne variant har flere mutationer i spidsproteinet, herunder K417N, E484K, N501Y. Den har potentiel reduktion i neutralisering ved nogle EUA monoklonale antistofbehandlinger.

SARS-CoV-2-epidemien i Brasilien blev domineret af to stammer betegnet som P.1 og P.2, der husede mutationer i det receptorbindende domæne af spidsproteinet (S). P.1 -stammen (kaldet Gamma) betragtes som en variant af bekymring (VOC), fordi den har potentiale til reduceret neutralisering af nogle amerikanske monoklonale antistofbehandlinger. Denne stamme har flere mutationer i S-proteinet (inklusive K417T, E484K, N501Y), og dets fremkomst er blevet forbundet med en anden epidemisk bølge af COVID-19 i staten Amazonas. P.2 -stammen

betragtes som en variant under overvågning (VUM) og har kun E484K -mutationen. P.2 -stammen blev opdaget som den mest udbredte variant i flere stater i Amazonas over hele landet i slutningen af 2020 og begyndelsen af 2021.

Udseendet af varianter, der øger overførbarheden af virussen, dens virulens eller som undgår virkningen af neutraliserende antistoffer genereret efter den naturlige infektion eller vaccinen, udgør et første ordens folkesundhedsproblem, der kan have en vigtig indvirkning på kontrollen med pandemien. Af denne grund tillader VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit til BD MAX™ -systemet påvisning af HV 69/70-sletning, K417N eller K417T-mutationer associeret med alfa-, beta- og gamma-varianter af bekymring.

3. Procedurens princip

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System er designet til kvalitativ påvisning af RNA med HV 69/70-deletion, K417N-mutation og K417T-mutation i SARS-CoV-2 *S*-genet fra nasofaryngeale/orofaryngeale podepindsprøver og spytprøver. Detektionen foretages i et-trins realtids-RT-PCR-format, hvor den omvendte transskription og den efterfølgende forstærkning af den specifikke målsekvens finder sted i samme reaktionsrør. Det isolerede RNA-mål transkriberes, hvilket genererer komplementært DNA ved revers transkriptase, som efterfølges af amplifikationen af et bevaret område af *S*-genet for SARS-CoV-2 for HV 69/70-deletion, K417N-mutation og K417T-mutation ved hjælp af specifikke primere og fluorescerende -mærkede sonder.

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ Systemer baseret på 5´exonukleaseaktivitet fra DNA-polymerase. Under DNA-forstærkningen spalter enzym dette proben, som er bundet til den komplementære DNA-sekvens og adskiller quencher-farvestoffet fra rapportøren. Denne reaktion genererer en stigning i det fluorescerende signal, som er proportional med mængden på målskabelonen. Denne fluorescens måles af BD MAX™ System.

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ Systemindeholder i hvert rør alle de komponenter, der er nødvendige til realtids-PCR-analyse (specifikke primere/sonder, dNTPS, buffer, polymerase, omvendt transkriptase) i et stabiliseret format samt en endogen intern kontrol til overvågning af ekstraktionsprocessen og/eller hæmning af polymeraseaktiviteten. Analysen bruger et humant husholdningsgen som en endogen intern kontrol (IC) (humant *RNase P*-gen). Humane husholdningsgener er involveret i grundlæggende cellevedligeholdelse og forventes derfor at være til stede i alle nukleerede humane celler og opretholde relativt konstante ekspressionsniveauer.

Mål	Kanal	Gen
HV 69/70-deletion	475/520	*S*-gen
K417N-mutation	530/565	*S*-gen
K417T-mutation	585/630	*S*-gen
Endogen intern kontrol (IC)	630/665	Menneskelig t *RNase P*-gen

Tabel 1. Mål, kanal og gener.

4. Leverede reagenser

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System indeholder følgende materialer og reagenser, som er beskrevet i Tabel 2:

Reagens/Materiale	Beskrivelse	Farve/ Stregkode	Mængde
SARS-CoV-2 Variant reaction tube	En blanding af enzymer,primere sonder, buffere, dNTP'er, stabilisatorer og endogene interne kontroller i stabiliseret format	Grøn folie	2 poser med 12 transparente rør
Rehydration Buffer tube	Opløsning til rekonstitution af det stabiliserede produkt	11 folie	1 pose med 24 transparente rør

Tabel 2. Reagenser og materialer leveret i VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System med Kat. nr. VS-USB124 (444216).

5. Reagenser og udstyr, der skal leveres af brugeren

Følgende liste omfatter materialer og udstyr, der er nødvendige til brug, men ikke inkluderet VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System.

- Realtids-PCR-instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442827 eller 442828).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Mikropipetter (nøjagtighed mellem 2 og 1000 µl).
- Filterspidser.
- Pulverfrie engangshandsker
- Valgfrit: VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215)

6. Transport- og opbevaringsforhold

- Sættene kan sendes og opbevares ved 2 - 40 °C, indtil den udløbsdato, der er angivet på etiketten.
- Efter åbning af aluminiumsposerne, der indeholder reaktionsrørene, kan produktet bruges op til 28 dage.

7. Særlige forholdsregler for brugere

- Produktet er kun beregnet til brug af professionelle brugere, f.eks. laboratorie- eller sundhedspersonale og teknikere, der er uddannet i molekylærbiologiske teknikker.
- Til *in vitro*-diagnostisk brug.
- Brug ikke reagenser og/eller materialer, hvis udløbsdatoen er overskredet.
- Brug ikke sættet, hvis etiketten, der forsegler den ydre æske, er i stykker.
- Brug ikke reagenser, hvis beskyttelsesæsken er åben eller i stykker ved ankomsten.
- Brug ikke reagenser, hvis beskyttelsesposerne er åbne eller i stykker ved modtagelsen.

- Brug ikke reagenser, hvis tørremidlet ikke er til stede eller er i stykker inden i reagensposerne.
- Tørremidlet må ikke fjernes fra reagensposerne.
- Luk straks de beskyttende poser med reagenser med lynlåsforseglingen efter hver brug. Fjern eventuel overskydende luft i poserne inden forsegling.
- Brug ikke reagenser, hvis folien er blevet ødelagt eller beskadiget.
- Reagenser fra forskellige poser og/eller sæt og/eller partier må ikke blandes.
- Beskyt reagenser mod fugt. Længerevarende eksponering for fugt kan påvirke produktets ydeevne.
- Hold komponenterne væk fra lys.
- I tilfælde, hvor andre PCR-test udføres i det samme generelle område af laboratoriet, skal det sikres, VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, eventuelle yderligere reagenser, der er nødvendige til testen, og BD MAX™ System ikke er kontaminerede. Undgå altid mikrobiel og ribonuklease (RNase) / deoxyribonuklease (DNase) kontaminering af reagenser. Det anbefales at anvende sterile RNase/DNase-fri aerosolresistente engangspipettespidser eller positive fortrængningspipettespidser. Brug en ny spids til hver prøve. Handsker skal udskiftes før håndtering af reagenser og kassetter (BD MAX™ PCR Cartridge).
- For at undgå kontaminering af miljøet med amplikoner må BD MAX™ PCR Cartridge ikke brydes fra hinanden efter brug. Forseglingerne på BD MAX™ PCR Cartridge er designet til at forhindre kontaminering.
- Tilrettelæg en ensrettet arbejdsgang. Den skal begynde i ekstraktionsområdet og derefter flyttes til forstærknings- og detektionsområdet. Prøver, udstyr og reagenser må ikke returneres til det område, hvor det foregående trin blev udført.
- Følg god laboratoriepraksis. Brug beskyttelsestøj, engangshandsker, beskyttelsesbriller og maske. Man må ikke spise, drikke, ryge eller lægge makeup i arbejdsområdet. Vask hænder efter endt test.
- Prøverne skal behandles som potentielt smitsomme og/eller biologisk farlige, samt alle reagenser og materialer, der er blevet eksponeret for prøverne, og skal håndteres i overensstemmelse med de nationale sikkerhedsforskrifter. Træf de nødvendige forholdsregler under indsamling, opbevaring, behandling og bortskaffelse af prøver.
- Prøver og reagenser skal håndteres i et biologisk sikkerhedsskab. Anvend personlige værnemidler (PPE) i overensstemmelse med gældende retningslinjer for håndtering af potentielt smitsomme prøver. Affald bortskaffes i overensstemmelse med lokale retningslinjer.
- Regelmæssig dekontaminering af almindeligt anvendt udstyr anbefales, især mikropipetter og arbejdsflader.
- "I overensstemmelse med Forordning (EF) nr. 1907/2006 (REACH), kræver VIASURE Real Time PCR Detection Kits ikke materialesikkerhedsdatablade (Material Safety Data Sheets) som en del af deres klassificering som værende ufarlige for helbredet og miljøet, fordi de ikke indeholder stoffer og/eller blandinger, som opfylder kriterierne for fareklassificering iht. forordning (EF) nr. 1272/2008 (CLP), eller forefindes i koncentrationer, der er højere end den værdi, der er angivet i den nævnte forordning til deres erklæring.
- Hvis sættet bruges i kombination med VIASURE *SARS-CoV-2 (N1 + N2)* Real-Time PCR Detection Kit for BD MAX System (Ref: 444215), se den tilhørende brugsanvisning.
- Se brugervejledningen til BD MAX™ System for yderligere advarsler, forholdsregler og procedurer.

8. Analysemetode

8.1. Prøveindsamling, opbevaring og transport

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kitt til BD MAX™ System er blevet testet på nasopharyngeal podepinde og spytprøver, begge opsamlet i viralt transportmedium (VTM) - Vircell S.L. -; BD™ Universal Viral Transport (UVT) System media - BD - eller IMPROVIRAL ™ Viral Preservative Medium (VPM) -Guangzhou Improve Medical Instruments Co. Ltd og oropharyngeal podninger opsamlet i viralt transportmedium (VTM) - Vircell. Andre typer prøver skal valideres af brugeren.

Prøveudtagning, opbevaring og transport skal vedligeholdes i overensstemmelse med de betingelser, der er valideret af brugeren. Samlet set skal åndedræts- og spytprøver indsamles og mærkes på passende vis i rene beholdere med eller uden transportmidler (afhængigt af prøvetype) og behandles så hurtigt som muligt for at garantere testens kvalitet. Prøverne skal transporteres ved 2 til 8 °C i op til 72 timer i henhold til lokale og nationale bestemmelser for transport af patogent materiale. Ved langtidstransport (mere end 72 timer) anbefaler vi forsendelse ved ≤-20 °C eller lavere. Det anbefales at anvende friske prøver til testen. Prøverne kan opbevares ved 2 til 8 °C i op til 72 timer eller nedfryses ved -20 °C eller ideelt ved -70 °C for konservering. Gentagne fryse-tø-cyklusser bør undgås for at forhindre nedbrydning af prøven og nukleinsyrer.

De nasofaryngeale/orofaryngeale podepindsprøver og spytprøverne skal indsamles, transporteres og opbevares i overensstemmelse med de relevante laboratorieretningslinjer. For yderligere oplysninger henvises til CDC guideline (CDC-retningslinjer for prøveudtagning. Websted https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf and Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Websted https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html) og IDSA-retningslinje (Miller, J. M., Binnicker, M. J., Campbell, S.,... & Pritt, B. S. (2018). En vejledning i anvendelse af mikrobiologilaboratoriet til diagnosticering af smitsomme sygdomme: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Kliniske infektionssygdomme*, 67(6), e1-e94).

8.2. Prøveklargøring og RNA-ekstraktion

Udfør prøveforberedelsen i overensstemmelse med anbefalingerne i brugsanvisningen til det anvendte ekstraktionssæt, BD MAX™ ExK™ TNA-3. Bemærk, at nogle andre prøver kan kræve forbehandling. Brugeren skal udvikle og validere ekstraktions- og præparationsprocedurer, der er specifikke til formålet.

Når du bruger prøver fra nasopharyngeal eller oropharyngeal podning:

1. Pipette mellem 400 og 750 µl nasofaryngeale/orofaryngeale podepinde opsamlet i virale transportmedier (VTM) eller i BD™ Universal Viral Transport (UVT) systemmedier ind i et BD MAX™ ExK™ TNA-3 Sample Buffer Tube og luk røret med en septumhætte. Der sikres fuldstændig blanding ved at vortexe prøven ved høj hastighed i 1 minut. Fortsæt til BD MAX™ System Operation.

I tilfælde af spytprøver, der skal indsamles i transportmedier:

1. Spytprøver kan opsamles i Viral Transport Medium (VTM), BD™ Universal Viral Transport (UVT) eller IMPROVIRAL ™ Viral Preservative Medium (VPM) i et forhold på 1:3 (spyt:medie). Vortex i 1 minut ved høj hastighed. Der pipetteres 750 µl over i et BD MAX™ ExK™ TNA-3 Sample Buffer Tube, og røret lukkes med en septumhætte.

Der sikres fuldstændig blanding ved at vortexe prøven ved høj hastighed i 1 minut. Fortsæt til BD MAX™ System Operation.

Hvis der anvendes rene spytprøver:

1. Kombiner spyt med viralt transportmedium (VTM), BD™ Universal Viral Transport (UVT) eller IMPROVIRAL™ Viral Preservative Medium (VPM), så det endelige forhold mellem spyt:medie er 1:3. Vortex i 1 minut ved høj hastighed. Derefter pipetteres 750 µl opløsning over i et BD MAX™ ExK™ TNA-3 Sample Buffer Tube og røret lukkes med en septumhætte. Der sikres fuldstændig blanding ved at vortexe prøven ved høj hastighed i 1 minut. Fortsæt til BD MAX™ System System Operation.

8.3. PCR-protokol

Bemærk: Der henvises til brugervejledningen til BD MAX™ System for at få detaljerede instruktioner.

8.3.1. Oprettelse af PCR-testprogram til VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System

Bemærk: Hvis du allerede har oprettet testen for VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System kan du springe trin 8.3.1 over og gå direkte til 8.3.2.

1) Vælg fanen "Test Editor" (Testredigering) på skærmen "Run" (Kør) på BD MAX™ System
2) Klik på knappen "Create" (Opret).
3) I fanen Grundlæggende oplysninger i vinduet "Test Name" (testnavn) skal du navngive din test: VIASURE *SARS-CoV-2 Variant*.
4) I rullemenuen "Extraction Type" (Ekstraktionstype), vælg "ExK TNA-3".
5) Vælg "Type 5" i rullemenuen "Master Mix Format".
    a. Bemærk: Produktet kan bruges i kombination med VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215), vælg derefter "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)". (Dual Master Mix Koncentreret Lyofiliseret MM med Rehydratiseringsbuffer, Type 5)
6) I "Sample extraction parameters" (Parametre for prøveekstraktion) vælges "User defined" (Brugerdefineret), og prøvevolumen justeres til 950 µl.
7) I "Ct Calculation" (Ct-beregning) vælges "Call Ct at Threshold Crossing" (Beregn Ct når tærsklen krydses).
8) Hvis du kører softwareversion 5.00 eller nyere, skal du vælge følgende konfiguration i "Custom Barcodes" (Brugerdefinerede stregkoder):
    a. "Snap-In 2 Barcode" (Snap-In 2 stregkode): efterlad tom (vedrørende *SARS-CoV-2 Variant* reaction tube (reaktionsrør), der kræves ingen stregkodekonfiguration).
    b. "Snap-In 3 Barcode" (Snap-In 3 stregkode): 11 (vedrørende rehydration buffer tube (rehydratiseringsbufferrør)).
    c. "Snap-In 4 Barcode" (Snap-In 4 stregkode): 1G, hvis det bruges i kombination med *SARS-CoV-2 (N1 + N2)* reaction tube (reaktionsrør) og formatet"Dual Master mix Concentrated Lyophilized MM

with rehydration Buffer (Type 5)"(Dual Master Mix Koncentreret Lyofiliseret MM med Rehydratiseringsbuffer, Type 5) (Afsnit 8.3.1).

9) Indtast følgende parametre på fanen "PCR settings" (PCR-indstillinger): "Channel Settings" (Kanalindstillinger), "Gains" (Stigninger) og "Threshold" (Tærskel) (Tabel 3).
   a. Bemærk: Produktet kan bruges i kombination med VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System (Ref: 444215), "PCR Settingsr" og "Test Steps" skal udfyldes for Snap-In 4 (blå) position (se den tilsvarende brugsanvisning).

Channel (Kanal)	Alias (Alias)	Gain (Gevinst)	Threshold (Tærskel)	Ct Min (Ct Min)	Ct Max (Ct Max)
475/520 (FAM)	HV69-70	80	150	0	40
530/565 (HEX)	K417N	80	150	0	40
585/630 (ROX)	K417T	80	150	0	40
630/665 (Cy5)	IC	80	150	0	35
680/715 (Cy5.5)	-	0	0	0	0

Tabel 3. PCR setting (PCR-indstillinger).

Bemærk: Det anbefales at angive minimumsgrænseværdierne angivet ovenfor for hver kanal som udgangspunkt, men de endelige indstillinger bør bestemmes af slutbrugeren ved fortolkning af resultaterne for at sikre, at tærskler falder inden for eksponentiel fase af fluorescenskurverne og over ethvert baggrundsignal. Tærskelværdien for forskellige instrumenter kan variere på grund af forskellige signalintensiteter.

10) I fanen "PCR settings" (PCR-indstillinger) indtastes følgende parametre samt "Spectral Cross Talk" (Spektral krydstale) (tabel 4).

		False Receiving Channel (Falsk modtagekanal)				
	Channel (Kanal)	475/520	530/565	585/630	630/665	680/715
Excitation Channel (Excitationskanal)	475/520	-	3,0	0,0	0,0	0,0
	530/565	1,0	-	0,0	0,0	0,0
	585/630	0,0	0,0	-	0,0	0,0
	630/665	0,0	0,0	5,0	-	0,0
	680/715	0,0	0,0	0,0	0,0	-

Tabel 4. Parametre for "Spectral Cross Talk" (spektral krydstale).

11) Indtast PCR-protokollen (tabel 5) på fanen "Test Steps" (Testtrin).

Step Name (Trinnavn)	Profile Type (Profiltype)	Cycles (Cyklusser)	Time (s) (Tid(er))	Temperature (Temperatur)	Detect (Registrering)
Reverse transcription (Revers transskription)	Hold	1	900	45 °C	-
Initial denaturation (Indledende denaturering)	Hold	1	120	98 °C	-
Denaturation and Annealing/Extension (Data collection) (Denaturering og annotering/udvidelse (dataindsamling))	2-temperatur	45	10	95 °C	-
			61,1	63 °C	✓

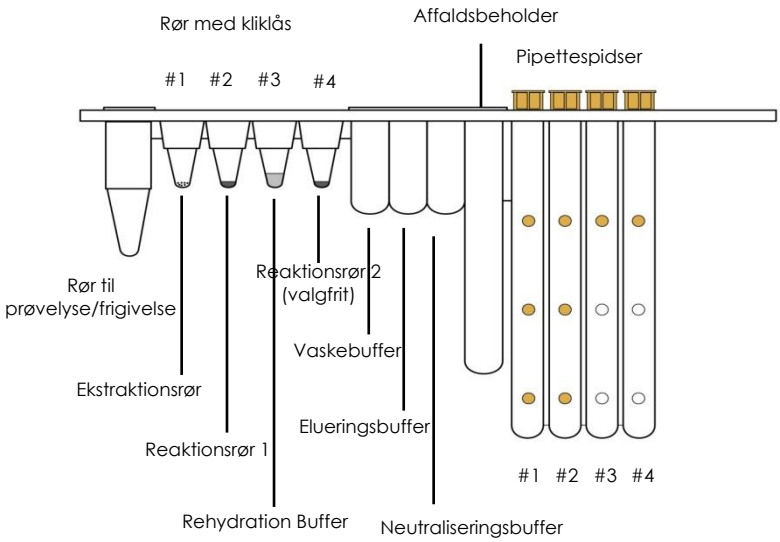
Tabel 5. PCR-protokol.

12) Klik på knappen "Save Test" (Gem test).

8.3.2. Opsætning af BD MAX™-stativ

1) For hver prøve, der skal testes, fjernes en samlet reagensstrimmel fra BD MAX™ ExK™ TNA-3 kit. Bank forsigtigt hver strimmel mod en hård overflade for at sikre, at alle væskerne ligger i bunden af rørene, og anbring dem i BD MAX™ Systems prøvestativer.
2) Fjern det nødvendige antal BD MAX™ ExK™ TNA Extraction Tubes (B4) (hvid folie) fra deres beskyttelsespose. Sæt udtræksrøret(-rørene) (hvid folie) i de tilsvarende positioner i TNA-strimlen (fastgør position 1, hvid farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk posen med lynlåsforseglingen.
3) Bestem og adskil det passende antal *SARS-CoV-2 Variant* reaction tube (reaktionsrør) (grøn folie) og klik ind i deres tilsvarende positioner i strimlen (Snap-position 2, grøn farvekodning på stativet. Se Figur 1).
    a. Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    b. Rehydreringen udføres korrekt ved at sørge for, at det frysetørrede produkt ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.
        i. Bemærk: Hvis du vælger formatet "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Dual Master Mix Koncentreret Lyofiliseret MM med Rehydratiseringsbuffer (type 5) (afsnit 8.3.1), skal du bestemme og adskille det passende antal yderligere *SARS-CoV-2* reaction tubes (reaktionsrør) (1G folie i tilfælde af VIASURE SARS -CoV-2 (N1 + N2) test) og klik ind i deres tilsvarende positioner i strimlen (Snap position 4, blå farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
4) Fjern det nødvendige antal Rehydration Buffer tubes (11 folie), og klik dem fast på deres tilsvarende pladser på strimlen (klik-position 3, ikke-farvet kodning på stativet. Se Figur 1). Fjern overskydende luft, og luk posen med lynlåsforseglingen.
    a. For at sikre, at overførslen udføres korrekt, skal man sørge for, at væsken ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt på hvert rør på en hård overflade for at sikre, at al bufferen er i bunden af røret.

Figur 1. BD MAX™ TNA Reagent Strip (TNA) fra BD MAX™ ExK ™ TNA-3 kit.



8.3.3. BD MAX™ Instrumentopsætning

1) Vælg fanen "Work List" (Arbejdsliste) på skærmen "Run" (Kør) på BD MAX™ Systemsoftware v4.50A eller nyere.
2) I rullemenuen "Test" skal du vælge VIASURE *SARS-CoV-2 Variant* (hvis den ikke allerede er oprettet, se afsnit 8.3.1).
3) Vælg det relevante lotnummer for kittet (fremgår af ekstraktionskittets udvendige æske) fra rullemenuen (valgfrit).
4) Indtast "Sample Buffer Tube" (prøvebufferrørets) identifikationsnummer i vinduet "Sample tube" (Prøverør) på "Worklist" (Arbejdsliste), enten ved at scanne stregkoden med scanneren eller ved manuel indtastning.
5) Udfyld prøven/patient-id'et og/eller adgangsvinduet på arbejdslisten, og klik på knappen "Save" (Gem). Fortsæt, indtil alle Sample Buffer Tubes (prøvebufferrør) er indtastet. Sørg for, at prøve-/patient-id'et og Sample Buffer Tubes matcher nøjagtigt.
6) Anbring det klargjorte Sample Buffer Tube i BD MAX™ Rack(s) (stativet/stativerne).
7) Sæt stativet/stativerne i BD MAX™ System (stativ A er placeret i venstre side af BD MAX™ System og stativ B i højre side).
8) Anbring det nødvendige antal BD MAX™ PCR Cartridges i BD MAX™ System.
9) Luk lågen til BD MAX™ System.
10) Klik på "Start Run" (Start procedure) for at starte proceduren.

8.3.4. BD MAX™ rapport

1) Klik på knappen "Results" (Resultater) i hovedmenuen.
2) Dobbeltklik enten på din kørsel på listen, eller tryk på knappen "View" (Vis).
3) Klik på "Print" (Udskriv), vælg: "Run Details, Test Details and Plot…" (Kør detaljer, testdetaljer og tegn grafik).
4) Klik på knappen "Print or Export" (Udskriv eller eksportér) på skærmbilledet Run Reports (Kør rapporter).

9. Tolkning af resultater

For en detaljeret beskrivelse af, hvordan man analyserer data, se BD MAX™ Systems brugervejledning.

Analyse af VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System er beregnet til at blive udført som en refleks på prøver med positivt resultat for SARS-CoV-2 RNA. Hvis den bruges sammen med VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX ™ System på prøver af ukendt status for tilstedeværelse af SARS-CoV-2 RNA, henvises til brugsanvisningen for resultater fortolkning til bestemmelse af SARS-CoV-2 RNA-resultatet.

Analysen af data udføres som BD MAX™-software i overensstemmelse med producentens anvisninger. BD MAX™-softwaren rapporterer Ct-værdier og stigningskurver for hver detektorkanal for hver prøve, og testes på følgende måde:

- En Ct-værdi på 0 angiver, at der ikke blev beregnet nogen Ct-værdi af softwaren ved den angivne tærskelværdi (se tabel 3). En forstærkningskurve for prøven, der viser en Ct-værdi på "0", skal kontrolleres manuelt.

- Ct-værdien -1 angiver, at ingen forstærkningskurve er forekommet.

- Enhver anden Ct-værdi skal fortolkes i sammenhæng med forstærkningskurve og i overensstemmelse med retningslinjerne for tolkning af prøven som anført i Tabel 6.

Kontrollér, at det indvendige styresignal fungerer korrekt for amplifikationsblandingen. Desuden skal du kontrollere, at der ikke foreligger nogen rapport over BD MAX™ Systemfejl.

Resultaterne skal læses og analyseres ved hjælp af følgende tabel:

HV 69/70 sletningsmål (475/520)	K417N-mutationsmål (530/565)	K417T-mutationsmål (585/630)	Endogenous Internal Control (630/665)	Fortolkning
+	-	-	+/-[1]	**HV 69/70-deletion fundet [1]**
-	+	-	+/-[1]	**K417N-mutation fundet t [1]**
-	-	+	+/-[1]	**K417T-mutation fundet [1]**
+	+	-	+/-[1]	**HV 69/70-deletion og K417N mutation fundet [1]**
+	-	+	+/-[1]	**HV 69/70-deletion og K417T mutation fundet [1]**
-	+	+	+/-[1]	**K417N- og K417T- mutation fundet [1]**
+	+	+	+/-[1]	**HV 69/70-deletion, K417N-mutation og K417T-mutation fundet [1]**
-	-	-	+[1]	**HV 69/70-deletion, K417N-mutation og K417T-mutation ikke fundet [1]**
-	-	-	-[2]	**Resultatet Unresolved (uløst) (UNR) optræder under tilstedeværelse af hæmmere i PCR-reaktionen eller når der opstår et overordnet problem (der ikke rapporteres med en fejlkode) under prøvekørslen og/eller forstærkningstrinnene.[2]**
IND	IND	IND	IND	**Analyseresultatet er Indeterminate (ubestemmeligt) (IND). Skyldes en fejl i BD MAX™ System. Analyseresultat, der vises i tilfælde af en instrumentfejl, der knyttet til en fejlkode.**
INC	INC	INC	INC	**Analyseresultatet er Incomplete (ufuldstændigt) (INC). Skyldes en fejl i BD MAX™ System. Analyseresultatet vises, hvor en fuldstændig kørsel ikke kunne gennemføres.**

Tabel 6. Prøvefortolkning.

+: Der opstod forstærkning

-: Der opstod ingen forstærkning.

1 En prøve betragtes som positiv, hvis Ct-værdien er mindre end 40. Den endogene interne kontrol (IC) kan både vise et forstærkersignal eller intet forstærkersignal. Sommetider er IC-detektionen ikke nødvendig, fordi et højt kopinummer for målet kan forårsage præferenceamplifikation af målspecifikke nukleinsyrer.

2 I tilfælde af HV 69/70 -deletion, K417N -mutation og K417T -mutation er målrettet mod steder, skal IC vise et forstærkningssignal med Ct mindre end 35. Ct-værdien kan være meget variabel på grund af den endogene interne kontrol er et humant rengøringsgen, der bør være til stede i alle humane kerneceller i den oprindelige prøve. Hvis der mangler signal eller Ct -værdi ≥ 35 for den endogene interne kontrol, betragtes resultatet som 'uløst', og det er nødvendigt at teste igen.

Resumé af mutationer forbundet med følgende slægter til stede i de mest kendte varianter af bekymring (VOC):

Stammer	WHO-mærke	Mutationer i S-genet [1]		
		HV 69/70-deletion	K417N-mutation	K417T-mutation
B.1.1.7	Alpha	X	-	-
B.1.351	Beta	-	X	-
P.1	Gamma	-	-	X

Tabel 7. Resumé af mutationer forbundet med kendte varianter af bekymring (VOC).

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

Andre varianter kan præsentere HV 69/70-deletion og mutationer K417T og K417N, fordi de ikke er specifikke for de nævnte varianter.

Den endelige tildeling til en afstamning skal udføres ved at rækkefølge.

I tilfælde af et fortsat tvetydigt resultat anbefales det at gennemgå brugsanvisningen, den ekstraktionsproces, som brugeren anvender; til at verificere den korrekte ydeevne for hvert RT-qPCR-trin og gennemgå parametrene og kontrollere kurvens sigmoide form og fluorescensintensiteten.

Resultaterne af testen bør vurderes af en læge på baggrund af anamnese, kliniske symptomer og andre diagnostiske tests.

10. Begrænsninger i testen

- Resultaterne af testen bør vurderes af en læge på baggrund af anamnese, kliniske symptomer og andre diagnostiske tests.
- Selvom denne test kan bruges sammen med andre typer prøver er den blevet valideret med nasofaryngeale/orofaryngeale podepinde indsamlet i Viral Transport Medium(VTM).
- For god testydeevne skal det frysetørrede produkt være i bunden af røret og ikke klæbe til det øverste område af røret eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.
- Et udseende af reaktionsblandingen i stabiliseret format, som normalt findes i bunden af røret, forskelligt fra det sædvanlige (uden konisk form, inhomogent, mindre/større i størrelse og/eller farve forskellig fra hvidlig) ændrer ikke testens funktionalitet.
- Testens kvalitet afhænger af prøvens kvalitet; korrekt ekstraheret nukleinsyre fra luftvejsprøver skal ekstraheres.
- Denne test er en kvalitativ test og giver ikke kvantitative værdier eller angiver antallet af tilstedeværende organismer.
- Meget lave målniveauer under detektionsgrænsen kan påvises, men resultaterne er muligvis ikke reproducerbare.

- Der er mulighed for falske positive resultater på grund af krydskontaminering af SARS-CoV-2 RNA med HV 69/70-deletion, K417N-mutation eller K417T-mutation i *S* genet, enten prøver indeholdende høje koncentrationer af mål-RNA eller kontaminering på grund af PCR produkter fra tidligere reaktioner.
- De specifikke primer- og probekombinationer til påvisning af HV 69/70-deletion, K417N-mutation eller K417T-mutation, der anvendes i VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System, viser ikke signifikante kombinerede homologier med det humane genom, Menneskelig mikroflora eller andre coronavirus, som kan resultere i forudsigelig falsk positiv.
- Falsk-negative resultater kan skyldes flere faktorer og kombinationer heraf, herunder:
    - Forkerte metoder til indsamling, transport, opbevaring og/eller håndtering af prøver.
    - Forkerte behandlingsprocedurer (herunder RNA-ekstraktion).
    - Nedbrydning af det virale RNA under forsendelse/opbevaring og/eller behandling af prøver.
    - Mutationer eller polymorfismer i primer- eller sondebindingsområder kan påvirke påvisningen af nye eller ukendte SARS-CoV-2-varianter.
    - En virusmængde i prøven under detektionsgrænsen for analysen.
    - Tilstedeværelsen af RT-qPCR-hæmmere eller andre typer interfererende stoffer. Virkningerne af vacciner, antivirale terapeutiske midler, antibiotika, kemoterapeutika eller immunsuppressive lægemidler, der anvendes til at forebygge COVID-19 eller under behandlingen af infektionen, er ikke blevet evalueret.
    - Manglende overholdelse af brugsanvisningen og analyseproceduren.
- Nogle prøver kan undlade at udvise *RNase P*-amplifikationskurver på grund af lave humane celletal i den oprindelige kliniske prøve. Et negativt IC -signal udelukker ikke tilstedeværelsen af HV 69/70 -deletion, K417N -mutation eller K417T -mutation i en klinisk prøve
- Et positivt testresultat indikerer ikke nødvendigvis tilstedeværelsen af levedygtige vira og betyder ikke, at disse vira er smitsomme eller forårsager kliniske symptomer. Et positivt resultat indikerer imidlertid tilstedeværelsen af målvirussekvenser.
- Tilstedeværelsen af HV 69/70-deletionen er forbundet med Alpha-varianten (B.1.1.7-stamme), K417N-mutation med Beta-variant (B.1.351-stamme) og K417T-mutation med Gamma-variant (P.1-stamme), dog den endelige tildeling til en afstamning skal ske ved at sekventering.
- Negative resultater udelukker ikke tilstedeværelse af SARS-CoV-2 RNA på grund af denne analyse er beregnet til at blive brugt med positive SARS-CoV-2-prøver.
- Hvis der opnås uafklarede, ubestemte eller ufuldstændige resultater ved hjælp af VIASURE *SARS-CoV-2 Variant* Real-Time PCR Detection Kit for BD MAX™ System, kræves en ny test. Uløste resultater kan skyldes tilstedeværelsen af hæmmere i prøven eller forkert rehydrering af frysetørrede reaktionsblandingsrør. Hvis der opstår en instrumentfejl, kan det medføre ubestemmelige eller ufuldstændige resultater.

11. Kvalitetskontrol

VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System iindeholder en intern endogen kontrol (IC) i hvert reaktionsrør, der bekræfter korrekt teknikydelse.

12. Ydelseskarakteristika

12.1. Klinisk sensitivitet og specificitet

Den kliniske ydeevne af VIASURE *SARS-CoV-2 Variant* Real-Time PCR Detection Kit for BD MAX ™ System blev testet ved hjælp af kliniske respiratoriske prøver (nasopharyngeal podepinde) fra patienter med mistanke om luftvejsinfektion. Resultaterne var følgende:

	Center	Prøvetype	Arbejdsgang	Mål
1	CerTest Biotec S.L (Zaragoza, Spain)	nasopharyngeal podepind	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	HV 69/70-deletion
				K417T-mutation
				K417N-mutation

Tabel 8. Sted, prøvetype, arbejdsgang og mål.

Sand-positive og -negative værdier, falsk-positive og -negative værdier, følsomhed, specificitetsværdier for VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System blev beregnet i forhold til hver komparatoranalyse som vist i følgende tabel:

Center	Komparatoranalyse	Mål	TP	TN	FP	FN	Følsomhed	Specificitet
1	Molekylær test TaqPath COVID-19 CE-IVD RT-PCR Kit / VIASURE *SARS-CoV-2* Real Time PCR Detection Kit+ sekventering	HV 69/70-deletion	48	167	0	2	96% (85 – 99)	100% (97 – 100)
		K417T-mutation	50	167	0	0	100% (91 – 100)	100% (97 – 100)
		K417N-mutation	7	209	0	1	88% (46 – 99)	100% (97 – 100)

Tabel 9. Ægte positive (TP) og negative (TN) værdier, falske positive (FP) og negative (FN) værdier, følsomhed, specificitet for VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System.

Resultatet viser enighed om at detektere HV 69/70 deletion, K417T og K417N SARS-CoV-2 mutationer ved hjælp af VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System.

For at vurdere kompatibiliteten mellem forskellige prøvematricer (nasopharyngeal podning, oropharyngeal podning og nasopharyngeal/oropharyngeal podning i Viral Transport Medium (VTM) fra Vircell) blev der udført en kompatibilitetsundersøgelse. De opnåede resultater viste, at de tre forskellige prøvematricer var kompatible med VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytisk sensitivitet

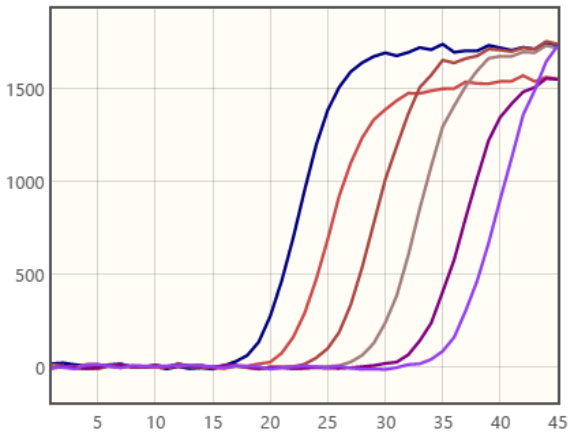
VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System detekteringsgrænse (LoD) -resultater med en positiv sats på ≥ 95% er som følger:

a) VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse (LoD) på ≥ 2 genomkopier/reaktion i nasopharynx-prøver og ≥ 5 genomkopier/reaktion i spytprøver til HV 69/70 eksklusion målt ved anvendelse af SARS-CoV-2 B.1.1.7-stammen.

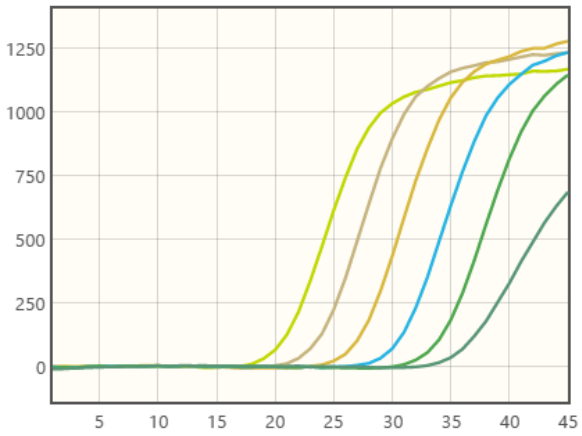
b) VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit til BD MAX ™ System har en detektionsgrænse (LoD) på ≥ 5 genom / reaktionskopier i nasopharynx-prøver og ≥ 5 genom/ reaktionskopier i spytprøver til K417N-mutation målt ved hjælp af SARS-CoV-2 B.1.351-stammen.

c) VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit til BD MAX ™ System har en detektionsgrænse (LoD) på ≥ 10 genom/reaktionskopier i nasopharynx-prøver og ≥ 15 genom/reaktionskopier i spytprøver til K417T-mutation målt ved hjælp af SARS-CoV-2 P.1-stammen.

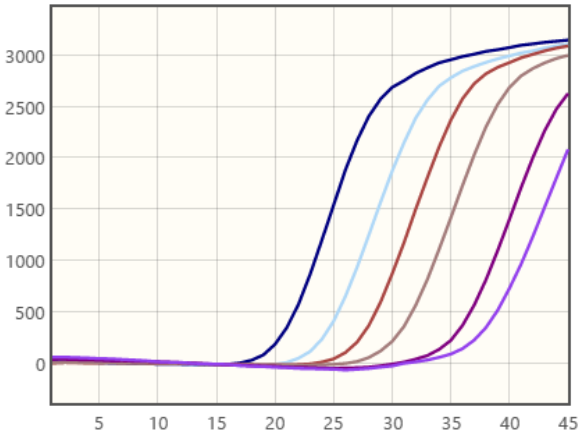
Figur 2. Fortyndingsserie af SARS-CoV-2 Variant (HV 69/70-deletion) (syntetisk cDNA) (5,3 * 10^5 -5,2 * 10^1 genomkopier pr. reaktion) kører på BD MAX™ System (475/520 (FAM) -kanalen) .



Figur 3. Fortyndingsserie af SARS-CoV-2 Variant (K417N-mutation) (syntetisk cDNA) (5,3*10^5 -5,2*10^1 genomkopier pr. reaktion) skabelon kørt på BD MAX ™ System (530/565 (HEX) -kanalen.



Figur 4. Fortyndingsserie af SARS-CoV-2-variant (K417T-mutation) (syntetisk cDNA) (5,3*10^5 -5,2*10^1 genomkopier pr. Reaktion) skabelon kørt på BD MAX™ System (585/630 (ROX) -kanalen).



12.3. Analytisk specificitet

Specificiteten af SARS-CoV-2 -analysen blev bekræftet ved at teste et panel bestående af forskellige mikroorganismer, der repræsenterer de mest almindelige respiratoriske patogener. Der blev ikke påvist krydsreaktivitet mellem nogen af følgende testede mikroorganismer:

Krydsreaktivitetstest					
Human Adenovirus-type 1-5, 8, 15, 31, 40 og 41	-	Influenza A/Michigan/45/2015 (H1N1)pdm09-virus	-	Mycoplasma pneumoniae	-
Bocavirus	-	Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09-virus	-	Mycobacterium tuberculosis	-
Bordetella bronchiseptica	-	Influenza A/Thüringen/5/17 (H3N2)-virus	-	Menneskelig parainfluenza 1, 2, 3 og 4 vira	-
Bordetella holmesii	-	Influenza A/Switzerland/9715293/2013 (H3N2)-virus	-	Pneumocytis jirovecii Type A1 og g885652	-
Bordetella parapertussis	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2)-virus	-	Menneskelig rhinovirus	-
Bordetella pertussis	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8)-virus	-	Respiratorisk syncytialvirus (RSV) A/B	-
Chlamydia caviae	-	Influenza A/Anhui/1/2013 (H7N9)-virus	-	SARS Coronavirus-stamme Frankfurt 1	-
Chlamydia psittaci genotype A og C	-	Influenza B/Brisbane/60/2008-virus	-	Menneskelig stamme 2019-nCoV BetaCoV/Tyskland/BavPat1/2020 s.1*	-
Chlamydophila pneumoniae CM-1	-	Influenza A/South Australia/55/2014, IVR-175	-	Menneskelig 2019-nCoV-stamme 2019-nCoV/Italy-INMI1*	-
Menneskelig coronavirus 29E OC43, NL63 og HKU1	-	Influenza B/Phuket/3073/2013 virus	-	MT007544.1 (SARS-CoV-2 isolat Australia/VIC01/2020)*	-
MERS Coronavirus	-	Influenza B/Florida/04/06-virus	-	MN908947.3 (SARS-CoV-2 isolat Wuhan-Hu-1)*	-
Enterovirus Coxsackievirus A24, A9 og B3	-	Legionella bozemanii	-	SARS-CoV-2 stamme 2019nCoV/USAWA1/2020*	-
Enterovirus Echovirus 30	-	Legionella dumoffii	-	SARS-CoV-2 BetaCoV/Berlin/ChVir1670/2020_IsolatBER*	-
Enterovirus 68 , 71	-	Legionella longbeachae	-	SARS-CoV-2 BetaCoV/Munich/ChVir984/2020*	-
Haemophilus influenzae MinnA	-	Legionella micdadei	-	SARS-CoV-2 BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER*	-
Influenza A/New Caledonia/20/99(H1N1)-virus	-	Legionella pneumophila	-	Staphylococcus aureus	-
Influenza A/Victoria/210/2009 (H3N2)	-	Menneskelig metapneumovirus A og B	-	Streptococcus pneumoniae	-
Influenza A/California/7/2009(H1N1)pdm09 virus	-	Moraxella catarrhalis	-	Streptococcus pyogenes	-

Tabel 10. Referengcepatogene mikroorganismer, der blev brugt i denne undersøgelse.

*Bemærk, at detektionen af disse SARS-CoV-2-stammer ikke overvejes i denne analyse. Denne test er designet til kvalitativ påvisning af HV 69/70-deletionen, K417N-mutationen og K417T-mutationen i *S*-genet til stede i SARS-CoV-2 Alpha-, Beta- og Gamma-varianterne (B.1.1.7-, B.1.351- og S. 1-stammer), blandt andre.

12.4. Analytisk reaktivitet

Reaktiviteten af VIASURE *SARS-CoV-2 Variant* Real Time PCR Detection Kit for BD MAX™ System blev evalueret mod syntetiske RNA-kontroller for to forskellige sekvenser associeret med Alpha-varianten (B.1.1.7_710528 UK Variant og B.1.1.7_601443 UK Variant), en sekvens, der er knyttet til Beta-varianten (Control 16, SARS-CoV-2 stamme B.1.351 South Africa/KRISP-ECK005299/2020) og en sekvens, der er knyttet til gamma-varianten (kontrol 17, SARS-CoV-2 slægt P.1 Japan/Brasilian variant Japan/IC-0564/2021), viser positive resultater.

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Symbols for IVD components and reagents/ Symboler for IVD-komponenter og -reagenser

IVD	*In vitro* diagnostic device *In vitro*-diagnostisk udstyr	Keep dry Opbevares tørt	Use by Anvendes inden	Manufacturer Producent	LOT	Batch code (Lot) Batch-kode (parti)	
	Consult instructions for use Se brugsanvisningen	Temperature limitation Temperaturbegrænsning	Contains sufficient for <n> test Indeholder nok til <n> tests	DIL	Sample diluent Prøvefortynding	REF	Catalognumber Katalognummer

Trademarks

BD MAX™ is a registered trademark of Becton, Dickinson and Company.

Change Control / Ændringskontrol		
Version No. / Versionsnr.	**Changes / Ændringer**	**Date / Dato**
00	Original version / Original version.	10/08/2021

Table A 2. Control change table/ Tabel over ændringskontrol.

Revision: 10th August 2021

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