VIASURE

Real Time PCR Detection Kit

SARS-CoV-2 (N1 + N2)

for BD MAX™ System

CE IVD

These instructions for use apply to the following reference / *Disse brugsanvisninger gælder for følgende reference:*

PRODUCT / PRODUKT	REFERENCE / REFERENCE
VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System	444215 / VS-NCO324

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

Content

Indhold

ENGLISH

1. Intended use

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System is an automated real-time RT-PCR test designed for the qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal/oropharyngeal swab and saliva samples from individuals suspected of Coronavirus disease 2019 (COVID-19) by their healthcare professional (HCP). This test is intended to be used as an aid in the diagnosis of COVID-19 in combination with clinical and epidemiological risk factors. 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 SARS-CoV-2.

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 producing 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].

3. Principle of the procedure

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System is designed for the qualitative detection of RNA from SARS-CoV-2 in nasopharyngeal/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 two conserved regions of *N* gene (N1 and N2) using specific primers and fluorescent-labeled probes.

VIASURE *SARS-CoV-2 (N1 + N2)* 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 (N1 + N2)* 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
SARS-CoV-2	475/520	*N* gene (N2 region)
SARS-CoV-2	630/665	*N* gene (N1 region)
Endogenous Internal Control (IC)	530/565	human *RNase P* gene

Table 1. Target, channel and genes.

4. Reagents provided

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System includes the following materials and reagents detailed in Table 2:

Reagent/Material	Description	Barcode	Amount
SARS-CoV-2 (N1 + N2) reaction tube	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and endogenous internal control in stabilized format	1G 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 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System with Cat. N°. VS-NCO324 (444215).

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 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

- Real-time PCR instrument: BD MAX™ System.
- 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.

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 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 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 (N1 + N2)* 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.
- 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 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System has been tested on nasopharyngeal/ oropharyngeal swabs collected in viral transport media (VTM) (Vircell S.L., Spain); nasopharyngeal swabs collected in BD™ UVT System media, Virus transport and preservation medium (Biocomma®), UTM Viral transport (COPAN, Diagnostic Inc.), sterile transport medium (Deltalab®), Universal transport medium (UTM) and IMPROVIRAL™ Viral Preservative Medium (VPM) from Guangzhou Improve Medical Instruments Co. Ltd; and saliva samples collected in Viral Transport Medium (VTM), BD™ Universal Viral Transport (UVT) or IMPROVIRAL™ Viral Preservative Medium (VPM). 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/ 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 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System

Note: If you have already created the test for the VIASURE *SARS-CoV-2 (N1 + N2)* 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 (N1 + N2)*.

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 an additional VIASURE for BD MAX™ test, 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: 1G (concerning *SARS-CoV-2 (N1 + N2)* reaction tube).

   b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube).

   c. Snap-In 4 Barcode: another VIASURE reaction tube (different foil) if you choose 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 an additional VIASURE for BD MAX™ test, "PCR Settings" and "Test Steps" should be completed for Snap-In 2 (green) and Snap-In 4 (blue) positions.

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	SARS-CoV-2 N2 target	80	150	0	40
530/565 (HEX)	Endogenous IC	80	150	0	35
585/630 (ROX)	-	0	0	0	0
630/665 (Cy5)	SARS-CoV-2 N1 target	80	150	0	40
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	0.0	-	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	✓

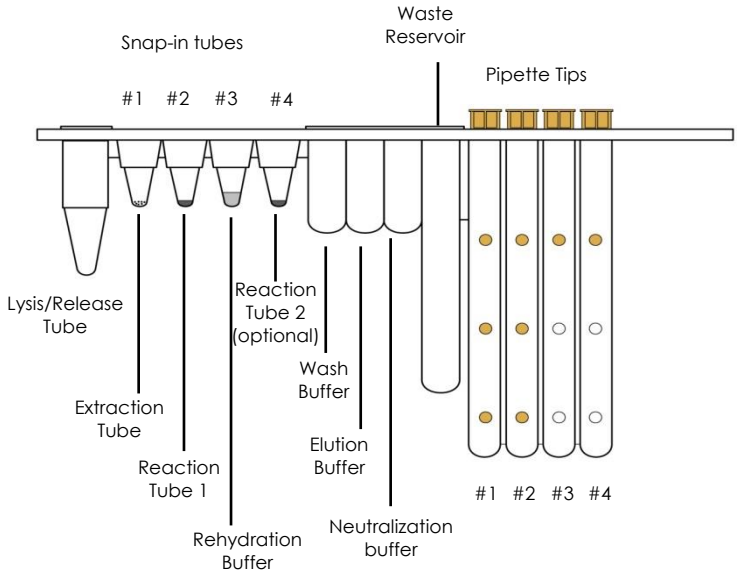
Table 5. PCR protocol.

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 (N1 + N2)* reaction tubes (1G 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 VIASURE reaction tubes (different foil) 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 (N1 + N2)* (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.

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:

SARS-CoV-2 (N2 target) (475/520)	Endogenous Internal Control (530/565)	SARS-CoV-2 (N1 target) (630/665)	Interpretation
+	+/-[1]	+	**SARS-CoV-2 *N gene* RNA Detected [1]**
+[2]	+/-[1]	-	**SARS-CoV-2 *N gene* RNA Detected [1,2]**
-	+/-[1]	+[2]	**SARS-CoV-2 *N gene* RNA Detected [1,2]**
-	+[3]	-	**SARS-CoV-2 *N* gene RNA Not Detected[3]**
-	-[3]	-	**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.[3]**
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	**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 If only one target site of the *N* gene amplifies, verify the sigmoid shape of the curve and the intensity of fluorescence. In case of a doubtful interpretation, depending on the available material, it is also recommended to:

a) re-extract and re-test another aliquot of the same specimen (if possible, increase sample volume to 750 µl) or,
b) obtain a new specimen and re-test.

3 In the case of SARS-CoV-2 target 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.

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 and oropharyngeal swabs and saliva samples, both collected in 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, 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 conserved regions of *N* gene used in VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System have been designed based on the US CDC assay for specific detection of SARS-CoV-2 by amplifying two unique regions of the *N* gene. They do not show significant combined homologies with the human genome, human microflora, SARS-CoV 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 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.
- A single-target site amplification or even random positive results is suggestive of slightly different amplification yield of the target sites of the *N* gene. Samples with low viral load might result in *N* single target amplification. In case of a doubt, it is recommended referring to a reference laboratory for further testing if clinically indicated.
- 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 SARS-CoV-2 RNA 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 (*N* genes).
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. The collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- If diagnostic tests for other respiratory illnesses are negative and the patient's clinical presentation and epidemiological information suggest that SARS-CoV-2 infection is possible, then a false negative result should be considered, and a re-testing of the patient should be discussed.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *SARS-CoV-2 (N1 + N2)* 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 (N1 + N2)* 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 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System was tested using respiratory clinical samples (nasopharyngeal swabs and oropharyngeal swabs) from patients with suspected respiratory infection. The results were as follows:

	Site	Sample type	Workflow	Target
1	Hospital Universitario Miguel Servet (HUMS)	nasopharyngeal swab	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	SARS-CoV-2
2	"Servicio de Microbiología" of the Hospital Universitario Marqués de Valdecilla (Santander, Spain)	nasopharyngeal swab	MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the KingFisher Flex System instrument (ThermoFisher) + BD MAX™ System	SARS-CoV-2

Table 7. Site, sample type, workflow and target.

True positive and negative values, false positive and negative values, sensitivity, specificity values for VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System were calculated in relation to each comparator assay as shown in the following tables:

Site	Comparator assay	Target	TP	TN	FP	FN	Sensitivity	Specificity
1	Simplexa™ COVID-19 Direct assay	SARS-CoV-2	63	189	2	0	100% (94-100)	99% (96-99)
	Cobas® SARS-CoV-2 real time RT-PCR test	SARS-CoV-2	16	58	2	0	100% (79-100)	96% (88-99)
	Allplex™2019-nCoV Assay	SARS-CoV-2	71	75	0	0	100% (94-100)	100% (95-100)
2	TaqPath COVID-19 CE-IVD RT-PCR Kit molecular assay + sequencing	SARS-CoV-2	99	0	0	0	100% (96-100)	n.a*

Table 8. True positive and negative values, false positive and negative values, sensitivity, specificity for VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

Due to the fact that negative samples were not analyzed, the calculation of the specificity of the test could not be performed.

In order to evaluate the compatibility of different sample matrices (nasopharyngeal swab, oropharyngeal swab and nasopharyngeal/oropharyngeal swab in VTM from Vircell), a compatibility study have been carried out. The obtained results showed that the three different sample matrices were compatible with the *SARS-CoV-2 (N1 + N2)* reaction tube.

The performance of VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System with saliva samples was evaluated. Negative saliva single samples spiked with a known concentration of frozen quantified heat-inactivated culture 2019 Novel Coronavirus, Strain:2019-nCoV/USA-WA-1/2020 (ATCC-VR-1986HK) were tested. The evaluation was designed to be carried out with 30 positive samples (20 samples 2 times LoD (2xLoD), equivalent to 0.53 genome copies (GC)/µL, and 10 samples 5 times LoD (5xLoD) equivalent to 1.32 genome copies (GC)/µL) and 10 negative samples. This assay was performed using a 750 µl sample volume of each condition added in the Sample Buffer Tube (SBT) of the TNA-3 Extraction Kit and it was run in full process mode (Automated extraction and PCR amplification) using BD MAX™ ExK™ TNA-3.

The percentage of agreement was calculated in relation to the expected result for each individual sample and results are showed in the following table.

Saliva sample	Agreement
Positive sample (2xLoD)	97.5%
Positive sample (5xLoD)	100%
Negative sample	100%

Table 9. Percentage of agreement of VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System with saliva samples.

In conclusion, saliva samples were compatible with *VIASURE SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

Results show high agreement to detect SARS-CoV-2 using VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytical sensitivity

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System has a detection limit of ≥ 5 genome copies per reaction on nasopharyngeal swabs and ≥10 genome copies per reaction on saliva samples with a positive rate of ≥ 95%.

Note: The detection limit on saliva samples has been calculated using a sample volume of 750 µL (dilution 1:3 in VTM).

Figure 2. Dilution series of SARS-CoV-2 (N1 + N2) ($9.9*10^4$-$9.9*10^0$ and $5.0*10^0$ genome copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).

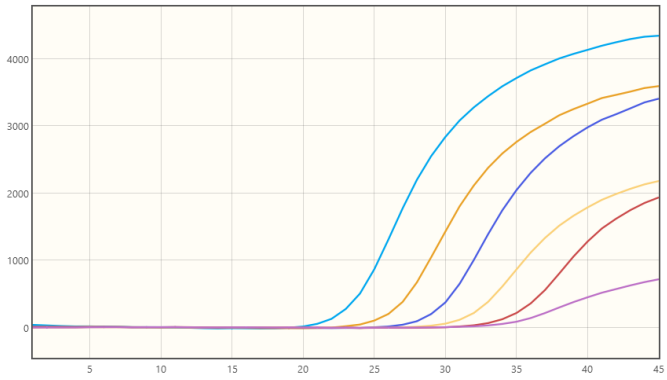
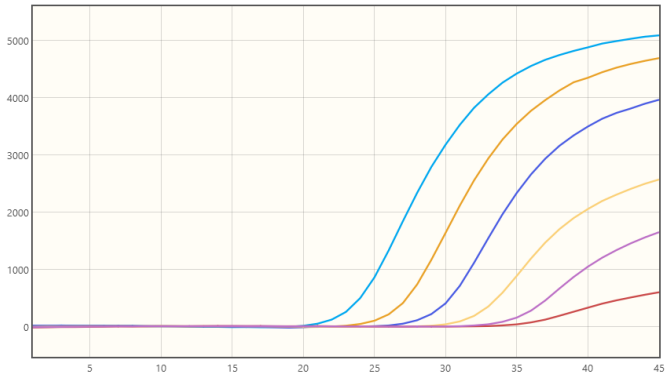


Figure 3. Dilution series of SARS-CoV-2 (N1 + N2) ($9.9*10^4$-$9.9*10^0$ and $5.0*10^0$ genome copies per reaction) template run on the BD MAX™ System (630/665 (Cy5) channel).



12.3. Analytical specificity

The specificity of the SARS-CoV-2 (N1 + N2) 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/New Caledonia/20/99(H1N1) virus	-	*Legionella longbeachae*	-
Human Bocavirus	-	Influenza A/California/7/2009(H1N1)pdm09 virus	-	*Legionella micdadei*	-
Bordetella bronchiseptica	-	Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-	*Legionella pneumophila*	-
Bordetella holmesii	-	Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-	Human metapneumovirus A and B	-
Bordetella parapertussis	-	Influenza A/Victoria/210/2009 (H3N2) virus	-	*Moraxella catarrhalis*	-
Bordetella pertussis	-	Influenza A/Thüringen/5/2017 (H3N2) virus	-	*Mycoplasma pneumoniae*	-
Chlamydia caviae	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-	*Mycobacterium tuberculosis* not rifampin resistant	-
Chlamydia psittaci genotype A and C	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-	Human parainfluenza 1, 2, 3 and 4 viruses	-
Chlamydophila pneumoniae CM-1	-	Influenza A/ South Australia/55/2014, IVR-175 (H3N2) virus	-	*Pneumocytis jirovecii* Type A1 and g885652	-
Human coronavirus 229E, OC43, NL63 and HKU1	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus	-	Human rhinovirus type C	-
MERS Coronavirus	-	Influenza A/Anhui/1/2013 (H7N9) virus	-	*Staphylococcus aureus* subsp. *aureus*	-
SARS Coronavirus Strain Frankfurt 1	-	Influenza B/Brisbane/60/2008 virus	-	*Staphylococcus epidermidis*	-
Enterovirus 68 and 71	-	Influenza B/Florida/04/06 virus	-	*Streptococcus pneumoniae Z022*	-
Enterovirus Echovirus 11 and 30	-	Influenza B/Phuket/3073/2013 virus	-	*Streptococcus pyogenes*	-
Enterovirus Coxsackievirus A24, A9 and B3	-	*Legionella bozemanii*	-	*Streptococcus salivarius*	-
Haemophilus influenzae MinnA	-	*Legionella dumoffii*	-	Respiratory syncytial virus (RSV) A and B	-

Table 10. Reference pathogenic microorganisms used in this study.

12.4. Analytical reactivity

The reactivity of VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System was evaluated against RNA from Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1, Human 2019-nCoV strain 2019-nCoV/Italy-INMI1, SARS-CoV-2 strain 2019nCoV/USA-WA1/2020, SARS-CoV-2 strain BetaCoV/Berlin/ChVir1670/2020_IsolatBER, SARS-CoV-2 strain BetaCoV/Munich/ChVir984/2020, SARS-CoV-2 strain BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER and synthetic RNA controls for four variants of the SARS-CoV-2 virus: SARS-CoV-2 isolate Australia/VIC01/2020, SARS-CoV-2 isolate Wuhan-Hu-1, B.1.1.7_710528 and and B.1.1.7_601443, showing positive results.

DANSK

1. Anvendelsesformål

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System er en automatiseret realtids-RT-PCR-test til kvalitativ påvisning af RNA fra SARS-CoV-2 i nasofaryngeale/orofaryngeale podepindsprøver og spytprøver fra personer, der mistænkes for at have coronavirus-sygdommen 2019 (COVID-19) af deres sundhedspersonale (HCP - healthcare professional). Denne test er beregnet til brug som et hjælpemiddel til diagnosen COVID-19 i kombination med kliniske og epidemiologiske risikofaktorer. Analysen anvender BD MAX™-systemet 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™-systemet. RNA ekstraheres fra prøver og komplementær DNA (cDNA) syntetiseres og forstærkes ved hjælp af RT-PCR og detekteres ved hjælp af fluorescerende rapportørfarveprober, der er specifikke for SARS-CoV-2.

2. Oversigt og forklaring

Coronavirus er indkapslede ikke-segmenterede positivt polariserede RNA-vira 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 eller 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 (to 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 og spytprøver 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].

3. Procedurens princip

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System er designet til kvalitativ påvisning af RNA fra SARS-CoV-2 i nasofaryngeale/orofaryngeale podepindsprøver og spytprøver. Detektionen foretages i et trins realtids RT-PCR-format, hvor den omvendte transkription og den efterfølgende forstærkning af den specifikke målsekvens finder sted i samme reaktionsrør. Det isolerede RNA-mål transkriberes og genererer komplementær DNA ved revers transkriptase, som efterfølges af forstærkning af et konserveret område af *N-gen* (N1 og N2) ved hjælp af specifikke primere og en fluorescensmærket probe.

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System er baseret på 5´exonukleaseaktivitet fra DNA-polymerase. Under DNA-forstærkningen spalter dette enzym 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™-systemet.

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System indeholder i hvert rør alle de komponenter, der er nødvendige til realtids-PCR-analyse (specifikke primere/sonder, dNTPs, buffer, polymerase, reverse-transkriptase) i et stabiliseret format samt en endogen intern kontrol til overvågning af ekstraktionsprocessen og/eller hæmning af polymeraseaktiviteten. Analysen anvender et humant husholdningsgen som et Endogent Internt Kontrolgen (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
SARS-CoV-2	475/520	*N-gen* (område N2)
SARS-CoV-2	630/665	*N-gen* (område N1)
Endogent Internt Kontrolgen (IC)	530/565	humant *RNase P-gen*

Tabel 1. Mål, kanal og gener.

4. Leverede reagenser

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System indeholder følgende materialer og reagenser, som er beskrevet i Tabel 2:

Reagens/Materiale	Beskrivelse	Stregkode	Mængde
SARS-CoV-2 (N1 + N2) reaction tube	En blanding af enzymer, primerprober, buffere, dNTP'er, stabilisatorer og endogent interne kontroller i stabiliseret format	1G folie	2 poser med 12 transparent rør
Rehydration Buffer tube	Opløsning til rekonstitution af det stabiliserede produkt	11 folie	1 pose med 24 transparent rør

Tabel 2. Reagenser og materialer leveret i VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System med kat. nr. VS-NCO324 (444215).

5. Reagenser og udstyr, der skal leveres af brugeren

Følgende liste omfatter materialer og udstyr, der er nødvendige til brug, men ikke inkluderet i VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

- Realtids-PCR-instrument: BD MAX™ System.
- 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.

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, som indeholder reaktionsrørene, kan de anvendes i op til 28 dage.

7. Særlige forholdsregler for brugere

- Produktet er tiltænkt professionelle brugere, såsom 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, at VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System, ExK™ TNA-3 extraction kit, eventuelle yderligere reagenser, der er nødvendige til testen, og BD MAX™-systemet ikke er kontaminerede. Undgå altid mikrobiel kontaminering og kontaminering af reagenser med ribonuklease (RNase)/deoxyribonuklease (DNase). 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 amplikationer må BD MAX™ PCR-kassetten 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 anvende kosmetiske produkter i arbejdsområdet. Vask hænder efter endt test.
- Prøverne skal behandles som potentielt smitsomme og / eller biofarligt 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, transport, opbevaring, håndtering 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.
- Se brugervejledningen til BD MAX™-systemet for yderligere advarsler, forholdsregler og procedurer.

8. Test procedure

8.1. Indsamling, opbevaring og transport af prøver

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System er blevet testet på nasofaryngeale/orofaryngeale podepindsprøver, der blev indsamlet i Viral Transport Medium (VTM) (Vircell S.L., Spain); nasofaryngeale podepindsprøver i BD™ UVT System media, Virus transport og preservation medium (Biocomma®), UTM Viral transport (COPAN, Diagnostic Inc.), sterile transport medium (Deltalab®), Universal transport medium (UTM) og IMPROVIRAL™ Viral Preservative Medium (VPM) fra Guangzhou Improve Medical Instruments Co. Ltd; og spytprøver indsamlet i Viral Transport Medium (VTM), BD™ Universal Viral Transport (UVT) eller IMPROVIRAL™ Viral Preservative Medium (VPM). 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 luftvejs- 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 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-retningslinjerne (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. Klargøring af prøver 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.

Ved brug af nasopharyngeal eller oropharyngeal prøver:

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

8.3. PCR-protokol

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

8.3.1. Oprettelse af PCR-testprogram for VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System

Bemærk: Hvis du allerede har oprettet testen til VIASURE *SARS-CoV-2 (N1 + N2)* 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™-systemet.
2) Klik på knappen "Create" (Opret).
3) Navngiv din test i fanen Basic Information (Grundlæggende oplysninger) i vinduet "Test Name" (Testnavn): dvs. VIASURE *SARS-CoV-2 (N1 + N2)*.
4) Vælg "ExK TNA-3" i rullemenuen "Extraction Type" (Ekstraktionstype).
5) Vælg "Type 5" i rullemenuen "Master Mix Format"
    a. Bemærk: Produktet kan bruges i kombination med en ekstra VIASURE til BD MAX™ test, og vælg derefter "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Dobbelt mastermix koncentreret frysetørret MM med rehydreringsbuffer (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-stregkode: 1G (vedrørende *SARS-CoV-2 (N1 + N2)* reaction tube)
    b. Snap-In 3-stregkode: 11 (vedrørende Rehydration Buffer tube)
    c. Snap-In 4-stregkode: et andet VIASURE-reaktionsrør (forskellig folie), hvis du vælger formatet "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (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 en ekstra VIASURE til BD MAX™ test, "PCR Settings" (PCR-indstillinger) og "Test Steps" testtrin skal udføres for Snap-In 2 (grøn) og Snap-In 4 (blå) positioner.

Channel (kanal)	*Alias* (Alias)	*Gain* (Gevinst)	*Threshold* (Tærskel)	*Ct Min* (Ct Min)	*Ct Max* (Ct Max)
475/520 (FAM)	SARS-CoV-2 N2-mål	80	150	0	40
530/565 (HEX)	Endogen IC	80	150	0	35
585/630 (ROX)	-	0	0	0	0
630/665 (Cy5)	SARS-CoV-2 N1-mål	80	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Tabel 3. PCR settings (PCR-indstillinger).

Bemærk: Det anbefales som udgangspunkt at fastsætte ovennævnte minimumstærskelværdier for hver kanal, men de endelige indstillinger skal fastlægges af slutbrugeren under resultatfortolkningen for at sikre, at tærskelværdierne falder inden for fluorescenskurvernes eksponentielle fase og over ethvert baggrundssignal. 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	0.0	-	0.0
	680/715	0.0	0.0	0.0	0.0	-

Tabel 4. Parametre for spektral krydstale *(Spectral cross-talk parameters)*.

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 (Omvendt transkription)	Hold	1	900	45°C	-
Initial denaturation (Indledende denaturering)	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection) (Denaturering og Annealing/Udvidelse (Dataindsamling))	2-temperatur	45	10	95°C	-
			61.1	63°C	✓

Tabel 5. PCR-protokol.

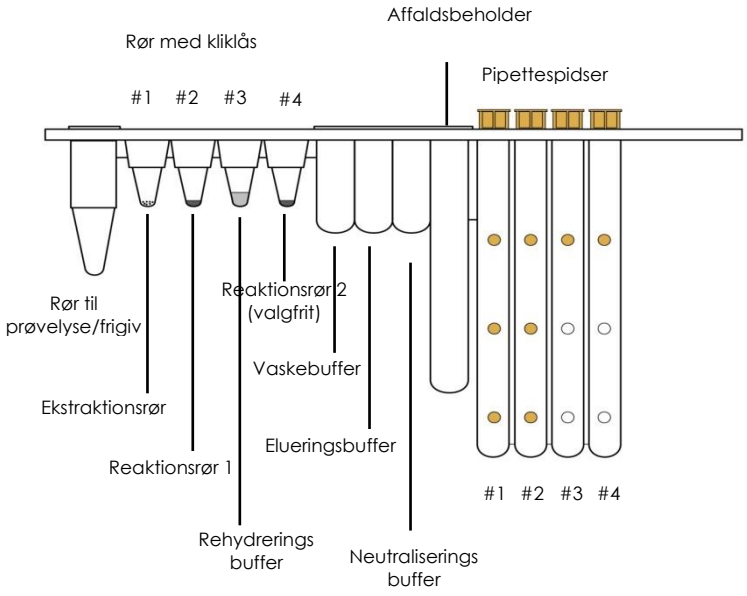
12) Klik på knappen "Save Test" (Gem test).

8.3.2. Opsætning af BD MAX™-stativ

1) For hver prøve, der skal testes, fjernes en Unitized Reagent-strimmel fra BD MAX™ ExK™ TNA-3 sættet. 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™-systemets 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 separer det relevante antal *SARS-CoV-2 (N1 + N2)* reaction tube (1G folie) og fastgør til 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 Lyophilised MM with Rehydration Buffer (Type 5)" (Dobbelt mastermix koncentreret frysetørret MM med rehydreringsbuffer (type 5)) (afsnit 8.3.1), bestemmes og adskilles det passende antal ekstra VIASURE reaktionsrør (forskellig folie) og klikkes fast i deres tilsvarende positioner i strimlen (klik-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 fastgør til deres tilsvarende positioner i strimlen (fastgørelsesposition 3, ikke-farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    c. 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 reagensstrimmel (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) Vælg VIASURE *SARS-CoV-2 (N1 + N2)* i rullemenuen "Test" (Test) (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 prøvebufferrørets identifikationsnummer i vinduet Sample tube (Prøverør) på Worklist (Arbejdsliste), enten ved at scanne stregkoden med scanneren eller ved manuel indtastning.
5) Udfyld vinduet Specimen/Patient ID og/eller Accession på Worklist (arbejdsliste), og klik på knappen "Save" (Gem). Fortsæt, indtil alle prøvebufferrør er indtastet. Sørg for, at prøve-/patient-id'et og prøvebufferrørene matcher nøjagtigt.
6) Anbring det klargjorte prøvebufferrør i BD MAX™-stativet/stativerne.
7) Sæt stativet/stativerne i BD MAX™-systemet (stativ A er placeret i venstre side af BD MAX™-systemet og stativ B i højre side).
8) Anbring det nødvendige antal BD MAX™ PCR Cartridges i BD MAX™-systemet.
9) Luk lågen til BD MAX™-systemet.
10) Klik på "Start Run" (Start procedure) for at starte procedure.

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 Eksporter) eller på skærmen "Run Reports" (Kør rapporter).

9. Tolkning af resultater

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

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 med 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:

SARS-CoV-2 (N2-mål) (475/520)	Endogent Internt Kontrolgen (530/565)	SARS-CoV-2 (N1-mål) (630/665)	Fortolkning
+	+/-[1]	+	**SARS-CoV-2 *N gene*-RNA påvist [1]**
+[2]	+/-[1]	-	**SARS-CoV-2 *N gene*-RNA påvist [1,2]**
-	+/-[1]	+[2]	**SARS-CoV-2 *N gene*-RNA påvist [1,2]**
-	+[3]	-	**SARS-CoV-2 *N* gene-RNA ikke påvist[3]**
-	-[3]	-	**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 prøvebehandlingen og/eller forstærkningstrinnene.[3]**
IND	IND	IND	**Analyseresultatet er Indeterminate (ubestemmeligt) (IND). Skyldes en fejl i BD MAX™-systemet. Analyseresultat, der vises i tilfælde af en instrumentfejl, der knyttet til en fejlkode.**
INC	INC	INC	**Analyseresultatet er Incomplete (ufuldstændigt) (INC). Skyldes fejl i BD MAX™-systemet. 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 Hvis kun ét af *N-genets* målområder forstærkes, skal man kontrollere kurvens sigmoide form og fluorescensens intensitet. I tilfælde af en tvivlsom fortolkning anbefales det afhængigt af det tilgængelige materiale også at:

a) ekstrahere og teste endnu en delprøve af samme prøve (om muligt øges prøvevolumen til 750 µl), eller
b) udtage en ny prøve og foretage en ny prøvning.

3 Hvis SARS-CoV-2-målområdet er negativt, 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 husholdningsgen, der bør være til stede i alle humane kerneceller i den oprindelige prøve. Hvis der ikke er signal eller en Ct-værdi ≥ 35 i den Endogent Internt Kontrol, betragtes resultatet som "uopklaret", og det er nødvendigt at teste igen.

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.
- Selv om denne test kan bruges sammen med andre typer prøver er den blevet valideret med nasofaryngeale/orofaryngeale podepindsprøver og spytprøverne begge indsamlet i VTM.
- For god testydeevne skal det frysetørrede produkt være i bunden af glasset og ikke klæbe til det øverste område af glasset 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 falsk positive resultater som følge af krydskontaminering med SARS-CoV-2, enten prøver indeholdende høje koncentrationer af mål-RNA eller kontaminering som følge af PCR-produkter fra tidligere reaktioner.
- De specifikke primer- og sondekombinationer til påvisning af bevarede områder af *N-genet*, der anvendes i VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System, er designet på grundlag af den amerikanske CDC-analyse til specifik påvisning af SARS-CoV-2 ved at forstærke to unikke områder af *N-genet*. De udviser ikke signifikante kombinerede homologier med det menneskelige genom, den menneskelige mikroflora, SARS-CoV eller andre coronavira, hvilket kan resultere i forudsigelige falsk-positive resultater.
- 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.
- En enkelt målsted amplifikation eller endda tilfældige positive resultater tyder på lidt anderledes amplifikationsudbytte af målstedet for *N-genet*. Prøver med lav virusmængde kan resultere i *N*-enkelt målamplifikation. I tvivlstilfælde anbefales det at henvise til et referencelaboratorium med henblik på yderligere testning, hvis det er klinisk indiceret.

- 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 SARS-CoV-2-RNA 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 (*N-gener*).
- Negative resultater udelukker ikke SARS-CoV-2-infektion og bør ikke anvendes som eneste grundlag for behandling eller andre beslutninger om patientbehandling. Optimale prøvetyper og tidspunktet for maksimale virusniveauer under infektioner forårsaget af SARS-CoV-2 er ikke fastlagt. Det kan være nødvendigt at indsamle flere prøver (typer og tidspunkter) fra samme patient for at påvise virusset.
- Hvis diagnostiske test for andre luftvejssygdomme er negative, og patientens kliniske præsentation og epidemiologiske oplysninger antyder, at SARS-CoV-2-infektion er mulig, bør et falsk negativt resultat overvejes, og en ny test af patienten bør drøftes.
- I tilfælde af at opnå uopklarede, ubestemte eller ufuldstændige resultater ved hjælp af VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System retesting vil være påkrævet. 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 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System indeholder en Endogen Intern Kontrol (IC) i hvert reaktionsrør, som bekræfter den korrekte udførelse af teknikken.

12. Ydelseskarakteristika

12.1. Klinisk semsitivitet og specificitet

Den kliniske præstation for VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System blev testet ved hjælp af nasofaryngeale podepinde og orofaryngeale podepind fra patienter med mistanke om luftvejsinfektion. Resultaterne var følgende:

	Sted	Prøvetype	Arbejdsgang	Mål
1	Hospital Universitario Miguel Servet (HUMS)	nasofaryngeale podepinde	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	SARS-CoV-2
2	"Servicio de Microbiología" Hospital Universitario Marqués de Valdecilla (Santander, Spain)	nasofaryngeale podepinde	MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit ved hjælp af KingFisher Flex System instrument (ThermoFisher) + BD MAX™ System	SARS-CoV-2

Tabel 7. Sted, prøvetype, arbejdsgang og mål.

Sand-positive og -negative værdier, falsk-positive og -negative værdier, følsomhed, specificitetsværdier for VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System blev beregnet i forhold til hver komparatoranalyse som vist i de følgende tabeller:

Sted	Komparatoranalyse	Mål	TP	TN	FP	FN	Sensitivitet	Specificitet
1	Simplexa™ COVID-19 Direct assay	SARS-CoV-2	63	189	2	0	100% (94-100)	99% (96-99)
	Cobas® SARS-CoV-2 real time RT-PCR test	SARS-CoV-2	16	58	2	0	100% (79-100)	96% (88-99)
	Allplex™2019-nCoV Assay	SARS-CoV-2	71	75	0	0	100% (94-100)	100% (95-100)
2	TaqPath COVID-19 CE-IVD RT-PCR Kit molecular assay + sequencing	SARS-CoV-2	99	0	0	0	100% (96-100)	n.a*

Tabel 8. Sand-positive eller -negative værdier, falsk-positive og -negative værdier, sensitivitet, specificitet til VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

Fordi negative prøver ikke blev analyseret, kunne beregningen af testens specificitet ikke udføres.

For at vurdere kompatibiliteten af forskellige prøvematricer (nasofaryngeal podepind, orofaryngeal podepind og nasofaryngeal/orofaryngeal podepind i VTM fra Vircell) blev der udført et kompatibilitetsstudie. De opnåede resultater viste, at de tre forskellige prøvematricer var kompatible med *SARS-CoV-2 (N1 + N2)* reaction tube (reaktionsrøret).

Ydeevnen for VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System med spytprøver blev evalueret. Negative enkeltprøver af spyt tilsat en kendt koncentration af frossen kvantificeret varmeinaktiveret kultur 2019 Novel Coronavirus, Stamme: 2019-nCoV/USA-WA-1/2020 (ATCC-VR-1986HK) blev testet. Evalueringen blev designet til at blive udført med 30 positive prøver (20 prøver 2 gange LoD (2xLoD), svarende til 0,53 genomkopier (GC)/µl, og 10 prøver 5 gange LoD (5xLoD) svarende til 1,32 genomkopier (GC)/µl) og 10 negative prøver. Denne analyse blev udført ved hjælp af et prøveeksemplarvolumen på 750 µl af hver tilstand, der blev tilføjet til Sample Buffer Tube (SBT) i TNA-3 Extraction Kit, og det blev kørt i fuld procestilstand (automatiseret ekstraktion og PCR-forstærkning) under anvendelse af BD MAX™ ExK™ TNA-3.

Procentdelen af overensstemmelse blev beregnet i forhold til det forventede resultat for hver enkelt prøve, og resultaterne vises i den nedenstående tabel.

Spytprøve	Overensstemmelse
Positiv prøve (2xLoD)	97.5%
Positiv prøve (5xLoD)	100%
Negativ prøve	100%

Tabel 9. Ydeevnen for VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System med spytprøver.

Det blev konkluderet, at spytprøver er kompatible med VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

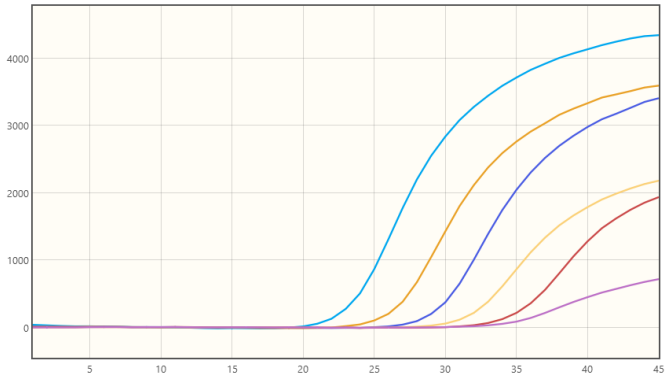
Resultaterne viser høj enighed om at detektere SARS-CoV-2 ved hjælp af VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytisk sensitivitet

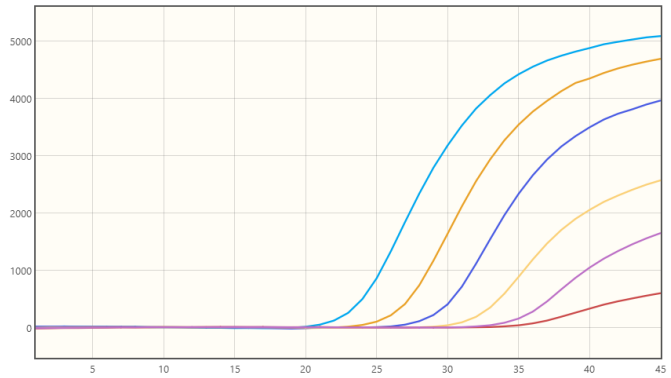
VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse på ≥ 5 genom-kopier pr. reaktion på nasofaryngeale podepindsprøver og ≥ 10 genom-kopier pr. reaktion i spytprøver med en positivrate på ≥ 95 %.

Bemærk: Detektionsgrænsen for spytprøver er beregnet ved hjælp af et prøvevolumen på 750 µl (fortynding 1:3 i VTM).

Figur 2. Fortyndingsserie af SARS-CoV-2 *(N1+N2)* (9,9*10⁴-9,9*10⁰ og 5,0*10⁰ genomkopier pr. reaktion) skabelon kørt på BD MAX™-systemet (475/520 (FAM)-kanalen).



Figur 3. Fortyndingsserie af SARS-CoV-2 *(N1+N2)* (9,9*10⁴-9,9*10⁰ og 5,0*10⁰ genomkopier pr. reaktion) skabelon kørt på BD MAX™-systemet (630/665(Cy5)-kanalen).



12.3. Analytisk specificitet

Specificiteten af SARS-CoV-2 *(N1 + N2)*-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					
Humant Adenovirus-type 1-5, 8, 15, 31, 40 og 41	-	Influenza A/New Caledonia/20/99(H1N1) virus	-	*Legionella longbeachae*	-
Humant Bocavirus	-	Influenza A/California/7/2009(H1N1)pdm09 virus	-	*Legionella micdadei*	-
Bordetella bronchiseptica	-	Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-	*Legionella pneumophila*	-
Bordetella holmesii	-	Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-	Humant metapneumovirus A og B	-
Bordetella parapertussis	-	Influenza A/Victoria/210/2009 (H3N2) virus	-	*Moraxella catarrhalis*	-
Bordetella pertussis	-	Influenza A/Thüringen/5/2017 (H3N2) virus	-	*Mycoplasma pneumoniae*	-
Chlamydia caviae	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-	*Mycobacterium tuberculosis* ikke rifampin-resistent	-
Chlamydia psittaci genotype A og C	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-	Humant parainfluenza 1, 2, 3 og 4 vira	-
Chlamydophila pneumoniae CM-1	-	Influenza A/ South Australia/55/2014, IVR-175 (H3N2) virus	-	*Pneumocytis jirovecii* Type A1 og g885652	-
Humant coronavirus 229E, OC43, NL63 og HKU1	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus	-	Humant rhinovirus type C	-
MERS Coronavirus	-	Influenza A/Anhui/1/2013 (H7N9) virus	-	*Staphylococcus aureus* subsp. *aureus*	-
SARS Coronavirus-stamme Frankfurt 1	-	Influenza B/Brisbane/60/2008 virus	-	*Staphylococcus epidermidis*	-
Enterovirus 68 og 71	-	Influenza B/Florida/04/06 virus	-	*Streptococcus pneumoniae Z022*	-
Enterovirus Echovirus 11 og 30	-	Influenza B/Phuket/3073/2013 virus	-	*Streptococcus pyogenes*	-
Enterovirus Coxsackievirus A24, A9 og B3	-	*Legionella bozemanii*	-	*Streptococcus salivarius*	-
Haemophilus influenzae MinnA	-	*Legionella dumoffii*	-	Respiratorisk syncytialvirus virus (RSV) A og B	-

Tabel 10. Reference patogene mikroorganismer, som anvendes i denne undersøgelse.

12.4. Analytisk reaktivitet

Reaktiviteten fra VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System blev evalueret mod RNA Humant 2019-nCoV-stamme BetaCoV/Germany/BavPat1/2020 p.1, Humant 2019-nCoV-stamme 2019-nCoV/Italy-INMI1, SARS-CoV-2-stamme 2019nCoV/USA-WA1/2020, SARS-CoV-2-stamme BetaCoV/Berlin/ChVir1670/2020_IsolatBER, SARS-CoV-2-stamme BetaCoV/Munich/ChVir984/2020, SARS-CoV-2-stamme BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER og syntetiske RNA-kontroller for fire varianter af SARS-CoV-2-virus: SARS-CoV-2-isolat Australia/VIC01/2020, SARS-CoV-2-isolat Wuhan-Hu-1, B.1.1.7_710528 og B.1.1.7_601443 viser positivt resultat.

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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 Batch-kode		Consult instructions for use Se brugsanvisning		Temperature limitation Temperaturbegrænsning	Σ	Contains sufficient for <n> tests Indeholder nok til <n> tests
DIL	Sample diluent Prøvefortynding	REF	Catalogue number Katalognummer				

Trademarks

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

Change Control / Ændringskontrol		
Version No. / Versions Nr.	**Changes / Ændringer**	**Date / Dato**
00	Original version/ Original version	21/05/2021

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

Revision: 21st May 2021

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