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 / *Ove upute za uporabu odnose se na sljedeću referencu:*

PRODUCT / PROIZVOD	REFERENCE / REFERENCA
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. / *Referenca za proizvod koji će se koristiti sa BD MAX™ sustavom.*

Content

Sadržaj

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	✓

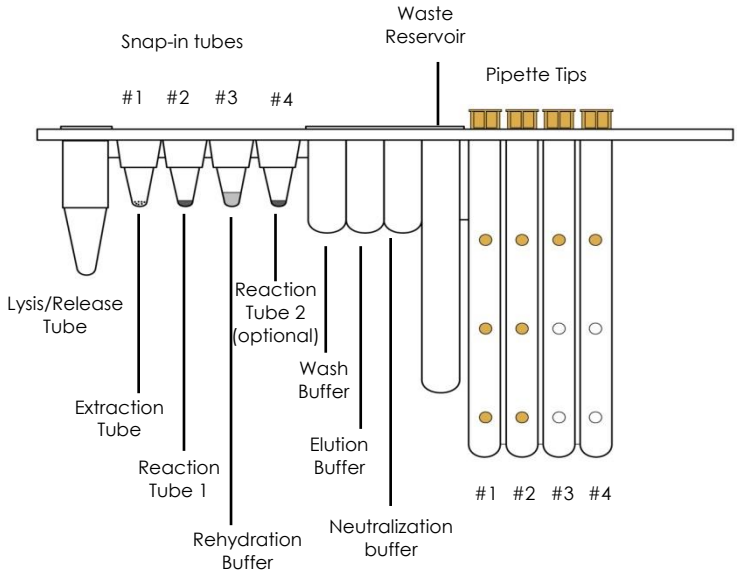
Table 5. PCR protocol.

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).

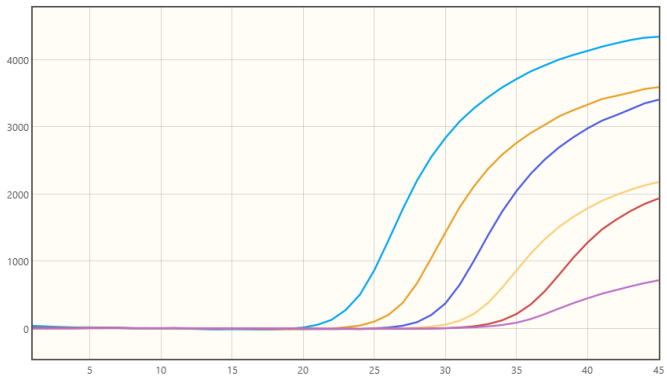
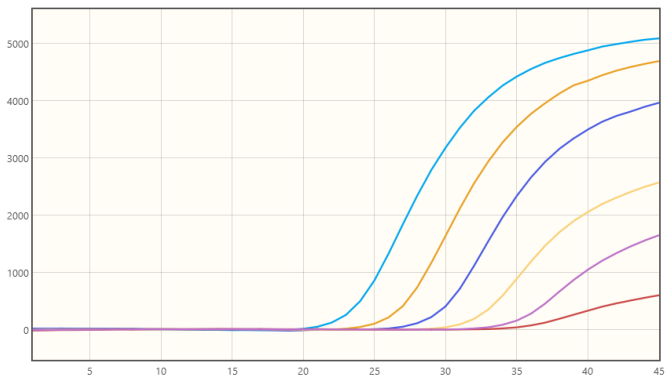


Figure 3. Dilution series of SARS-CoV-2 (N1 + N2) ($9.9*10^4$-$9.9*10^0$ and $5.0*10^0$ genome copies per reaction) template run on the BD MAX™ System (630/665 (Cy5) channel).



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 B.1.1.7_601443, showing positive results.

HRVATSKI

1. Namjena

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System je automatski RT-PCR test u stvarnom vremenu osmišljen za kvalitativnu detekciju RNK iz SARS-CoV-2 u nazofaringealnim/orofaringealnim brisevima i uzorcima sline od pojedinaca sa sumnjom na koronavirusnu bolest 2019 (COVID-19) od strane njihovih liječnika. Predviđeno je da se ovaj test koristi kao pomoć u dijagnosticiranju virusa COVID-19 u kombinaciji s kliničkim i epidemiološkim faktorima rizika. Test koristi BD MAX™ System sustav za automatiziranu ekstrakciju RNK, a zatim lančanu reakciju polimeraze RT-PCR u stvarnom vremenu, s priloženim reagensima u kombinaciji s univerzalnim reagensima i jednokratnim materijalom za BD MAX™ System sustav. RNK se ekstrahira iz uzoraka, komplementarna DNK (cDNK) se sintetizira i amplificira uporabom tehnike RT-PCR te detektira uporabom fluorescentne sonde za boju reporteta specifične za SARS-CoV-2.

2. Sažetak i objašnjenje

Koronavirusi su grupa ovijenih, nesegmentiranih, pozitivno usmjerenih RNK virusa koji spadaju u obitelj *Coronaviridae* [1,2]. Poznato je šest vrsta koronavirusa koji uzrokuju bolesti u ljudi [2]. Četiri virusa (229E, OC43, NL63 i HKU1) uzrokuju uobičajene simptome prehlade, a preostala dva (koronavirus teškog akutnog respiratornog sindroma (SARS-CoV) i koronavirus respiratornog sindroma Bliskog istoka (MERS-CoV)) su zoonotični i izazivaju teže komplikacije [2]. SARS-CoV i MERS-CoV prouzročili su preko 10.000 kumulativnih slučajeva u protekla dva desetljeća, sa stopama smrtnosti od 34% MERS-CoV i 10% SARS-CoV [1,3].

U prosincu 2019, nekoliko osoba koje su radile i živjele oko tržnice morskih plodova i životinja Huanan u Wuhanu, kineskoj pokrajini Hubei, oboljele su od upale pluća nepoznatog uzroka [2,4]. Analiza dubokog sekvenciranja respiratornih uzoraka ukazala je na novi koronavirus, koji je prvo dobio ime novi koronavirus 2019 (2019-nCoV), a zatim SARS-CoV-2 [5].

Potvrđen je prijenos SARS-CoV-2 s čovjeka na čovjeka, čak i tijekom inkubacijskog razdoblja bez simptoma, a virus uzrokuje teške respiratorne bolesti poput onih izazvanih virusom SARS-CoV [1,6,7,8]. Iako je upala pluća najčešća povezana bolest, u nekoliko bolesnika razvila se teška upala pluća, plućni edem, akutni respiratorni distres sindrom ili zakazivanje više organa i smrt [1,4]. Centri za kontrolu i prevenciju bolesti (Centers of Disease Control and Prevention, CDC) smatraju da se simptomi SARS-CoV-2 mogu pojaviti od 2 do 14 dana nakon izlaganja, pritom su najčešći zimica, kašalj, umor, anoreksija, mijalgija i dispneja [1,4,6,9]. Manje česti simptomi su grlobolja, začepljen nos, glavobolja, proljev, mučnina i povraćanje [1,4]. Prijavljeni su također i gubitak mirisa (anozmija) ili gubitak okusa (ageuzija) prethode nastupu respiratornih simptoma [9]. Stariji odrasli i osobe koje imaju teška postojeća medicinska stanja poput bolesti srca ili pluća ili dijabetesa pod većim su rizikom od razvoja ozbiljnijih komplikacija bolesti COVID-19 [10].

Dijagnosticiranje COVID-19 provodi se ranom detekcijom konvencionalnih uzroka upale pluća i detektira se sekvenciranjem sljedeće generacije ili metodama lančane reakcije polimeraze reverznom transkripcijom (RT-PCR) u stvarnom vremenu [1,11]. Trenutno je dostupno nekoliko testova koji detektiraju SARS-CoV-2 poput Kina CDC (ciljanje gena, *ORF1ab* i *N*), *Charité* – Njemačka (ciljanje gena, *RdRP* ili *E*) ili SAD CDC (dva cilja u *N* genu) [12].

Centar za kontrolu bolesti preporučuje uzorke iz gornjeg dijela dišnog trakta (nazofaringealni (NP) i orofaringealni (OP) brisevi, bris srednje turbine nosa, nazalni bris, uzorci nazofaringealnog ispirka/aspirata ili nazalnog

ispirka/aspirata (NW) i uzorci sline prikupljeni uglavnom od strane zdravstvenog radnika) i/ili uzorci donjeg dijela dišnog sustava (ispljuvak, endotrahealni aspirat ili bronhoalveolarna lavaža u bolesnika s težom bolešću dišnih putova) za identifikaciju SARS-CoV-2 [11]. Pored toga, mogu se prikupiti i drugi klinički uzorci poput krvi, mokraće i stolice za nadzor prisutnosti virusa [11,12].

3. Načelo postupka

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System mišljen je za kvalitativnu detekciju RNK iz SARS-CoV-2 u nazofaringealnim/orofaringealnim brisevima i uzorcima sline. Detekcija se obavlja u obliku lančane reakcije polimeraze reverznom transkripcijom (RT-PCR) u stvarnom vremenu koja se sastoji od jednog koraka, gdje se reverzna transkripcija i potom amplifikacija specifične ciljne sekvence vrši u istoj reakcijskoj epruveti. Nakon izolacije ciljne RNK, vrši se njena transkripcija čime se dobiva komplementarna DNK zahvaljujući reverznoj transkriptazi, nakon koje slijedi amplifikacija dvije konzervirane regije gena *N* (N1+N2) primjenom specifičnih početnica i fluorescentno obojane sonde.

VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System temelji se na aktivnosti *5´* egzonukleaze DNK polimeraze. Tijekom amplifikacije DNK ovaj enzim rascjepljuje sondu vezanu za sekvencu komplementarne DNK, čime se razdvaja supresorsku boju od indikacijskog gena. Ova reakcija dovodi do povećanja fluorescencijskog signala srazmjernog količini ciljnog predloška. Ta fluorescencija mjeri se na BD MAX™ sustavu.

Komplet za detekciju VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System sadrži u svakoj epruveti sve komponente potrebne za test PCR (specifične primere/sonde, dNTPs, pufer, polimerazu, reverznu transkriptazu) u stabiliziranom formatu, kao i endogenu unutarnju kontrolu za praćenje procesa ekstrakcije i/ili inhibicije aktivnosti polimeraze. Test koristi humani domaćinski gen kao endogenu internu kontrulu (IC) (humani *RNase P* gen). Ljudski domaćinski geni uključeni su u osnovno održavanje stanice te se stoga očekuje da će biti prisutni u svim ljudskim stanicama s jezgrom te održavati relativno stalne razine ekspresije.

Cilj	Kanal	Gen
SARS-CoV-2	475/520	*N* gen (N2 regija)
SARS-CoV-2	630/665	*N* gen (N1 regija)
Endogenu Internu Kontrulu (IC)	530/565	humani *RNase P* gen

Tablica 1. Cilj, kanal i geni.

4. Reagensi koji se isporučuju

Komplet za detekciju VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System uključuje sljedeće materijale i reagense detaljno opisane u Tablici 2:

Reagens/materijal	Opis	Crtični kod	Količina
SARS-CoV-2 (N1 +N2) reaction tube	Smjesa enzima, sondi za početnice, pufera, dNTP-ova, stabilizatora i endogene unutarnje kontrole u stabiliziranom obliku	Folija 1G	2 vrećice s 12 epruveta prozirno
Rehydration Buffer tube	Otopina za rekonstituciju stabiliziranog proizvoda	Folija 11	1 vrećica s 24 epruvete prozirno

Tablica 2. Reagensi i materijali osigurani u kompletu za detekciju VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System s Cat. N°. VS-NCO324 (444215).

5. Reagensi i oprema koju mora pribaviti korisnik

Sljedeći popis uključuje materijale i opremu koja je neophodna za korištenje, ali nije dio kompleta za detekciju VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

- Instrument za lančanu reakciju polimeraze (PCR) u stvarnom vremenu: BD MAX™ System.
- BD MAX™ ExK™ TNA-3 (Ref:442827 ili 442828).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Mikropipete (precizne u rasponu od 2 do 1000 µl).
- Nastavci za filtar.
- Nenaprašene jednokratne rukavice.

6. Uvjeti za transport i pohranu

- Kompleti se mogu transportirati i čuvati na temperaturi od 2-40°C do isteka roka valjanosti navedenog na naljepnici.
- Nakon otvaranja aluminijskih vrećica koje sadrže reakcijske epruvete mogu se iskoristiti u roku od 28 dana.

7. Mjere opreza za korisnike

- Proizvod je namijenjen isključivo uporabi od strane profesionalnih korisnika, kao što su laboratorijski ili zdravstveni djelatnici i tehničari, koji su prošli edukaciju o tehnikama molekularne biologije.
- Samo za *in vitro* dijagnostičku upotrebu.
- Nemojte koristiti reagense i/ili materijale kojima je istekao rok valjanosti.
- Nemojte koristiti komplet ako je potrgana naljepnica kojom je zatvorena vanjska kutija.
- Nemojte koristiti reagense ako je zaštitna kutija otvorena ili oštećena po prispijeću.
- Nemojte koristiti reagense ako su zaštitne vrećice otvorene ili oštećene po prispijeću.
- Nemojte koristiti reagense ako desikant u vrećicama s reagensima nije prisutan ili je oštećen.
- Nemojte vaditi desikant iz vrećica s reagensima.
- Nakon svake upotrebe odmah zatvorite zaštitne vrećice s reagensima pomoću patentnog zatvarača. Prije zatvaranja istisnite višak zraka iz vrećica.
- Nemojte koristiti reagense ako je folija potrgana ili oštećena.
- Nemojte miješati reagense iz različitih vrećica, kompleta i/ili serija.
- Zaštitite reagense od vlage. Duže izlaganje vlazi može utjecati na učinak proizvoda.
- Čuvajte komponente dalje od svjetlosti.
- U slučajevima gdje se drugi testovi PCR-om obavljaju u istom općem prostoru laboratorija potreban je oprez kako ne bi došlo do kontaminacije kompleta VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, svih dodatnih reagensa potrebnih za testiranje i BD MAX™ System. Svakako izbjegavajte kontaminaciju reagensa s mikrobima i ribonukleazama (Rnase)/deoksiribonukleazama (DNase). Preporučuje se uporaba jednokratnih, sterilnih vrhova volumetrijskih pipeta ili otpornih na aerosol, bez Rnase/Dnase. Koristite novi vrh sa svakim uzorkom. Rukavice se moraju promijeniti prije rukovanja reagensima i patronama (BD MAX™ PCR Cartridge).

- Radi sprječavanja kontaminacije okruženja amplikonima, nemojte odvajati BD MAX™ PCR Cartridge nakon uporabe. Brtve na BD MAX™ PCR Cartridge dizajnirane su za sprječavanje kontaminacije.
- Koristite jednosmjeran radni proces. Treba započeti u području za ekstrakciju, a zatim preći u područje za amplifikaciju i detekciju. nemojte vraćati uzorke, opremu i reagense u područje u kojem je obavljen prethodni korak.
- Pridržavajte se dobre laboratorijske prakse. Nosite zaštitnu odjeću, jednokratne rukavice, zaštitne naočale i masku. Nemojte jesti, piti pušiti niti nanositi kozmetičke proizvode u radnom prostoru. Operite ruke nakon što završite test.
- Uzorci se moraju smatrati potencijalno zaraznim i / ili biološki opasni, a isto vrijedi za reagense i materijale koji su bili izloženi uzorcima i mora se rukovati u skladu s nacionalnim sigurnosnim propisima. Poduzmite neophodne mjere opreza prilikom prikupljanja, prijevoz, pohrane, prijevoz i odlaganja uzoraka u otpad.
- Uzorcima i reagensima potrebno je rukovati u biološkom zaštitnom kabinetu. Koristite osobnu zaštitnu opremu (OZO) u skladu s važećim smjernicama za rukovanje potencijalno zaraznim uzorcima. Zbrinite otpad u skladu s lokalnim i državnim propisima.
- Preporučuje se redovita dekontaminacija opreme koja se često koristi, naročito mikropipeta i radnih površina.
- U skladu s Uredbom (EZ) br. 1907/2006. (REACH), kompleti „VIASURE Real Time PCR Detection Kits" ne zahtijevaju sigurnosne listove (Safety Data Sheets) zbog njihove klasifikacije kao neopasni za zdravlje i okoliš jer ne sadrže tvari i/ili smjese koje udovoljavaju kriterijima za razvrstavanje opasnosti dostupne u Uredbi (EZ) br. 1272/2008. (CLP) ili koje su u koncentracijama višim od vrijednosti utvrđene u spomenutoj uredbi za njihovo prijavljivanje.
- Posavjetujte se s Korisničkim priručnikom za BD MAX™ sustav u vezi s dodatnim upozorenjima, mjerama opreza i postupcima.

8. Postupak ispitivanja

8.1. Prikupljanje, pohrana i transport uzoraka

Komplet za detekciju VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System testiran je na nazofaringealnim/orofaringealnim brisevima prikupljenima u mediju Viral Transport Media (VTM) (Vircell S.L., Španjolska); na nazofaringealnim brisevima prikupljenima u mediju BD™ UVT System Media, Virus Transport and Preservation Medium (Biocomma®), UTM Viral Transport (COPAN, Diagnostic Inc.), Sterile Transport Medium (Deltalab®), Universal Transport Medium (UTM) i IMPROVIRAL™ Viral Preservative Medium (VPM) tvrtke Guangzhou Improve Medical Instruments Co. Ltd; te na uzorcima sline prikljupljenima u mediju Viral Transport Medium (VTM), BDTM Universal Viral Transport (UVT) ili IMPROVIRAL™ Viral Preservative Medium (VPM). Drukčije vrste uzoraka mora validirati korisnik.

Prikupljanje, pohrana i transport uzoraka treba obavljati u uvjetima koje je validirao korisnik. U globalu, respiratorne i sline uzorke treba prikupiti i označiti pravilno u čiste spremnike sa ili bez transportnog medija (ovisno o vrsti uzorka) i obraditi što je moguće prije u cilju osiguranja kvalitete testa. Uzorci se trebaju transportirati na temperaturi od 2 °C do 8 °C tijekom 72 sati u skladu s lokalnim i nacionalnim propisima za transport patogenih materijala. Za dugotrajni transport (duži od 72 sata) preporučujemo otpremanje na temperaturi od ≤-20 °C ili nižoj. Preporučuje se upotreba svježih uzoraka za test. Uzorci se mogu čuvati na temperaturi od 2 °C do 8 °C tijekom 72

sati ili držati smrznuti na temperaturi od -20°C ili idealno na -70°C za pohranu. Treba izbjegavati ponavljanje ciklusa smrzavanja i odmrzavanja kako bi se spriječilo propadanje uzorka i nukleinskih kiselina.

Nazofaringealni/orofaringealni brisevi i uzorci sline moraju se prikupljati, transportirati i pohraniti u skladu s odgovarajućim laboratorijskim smjernicama. Pojedinosti potražite u smjernicama Centara za prevenciju i kontrolu bolesti CDC (Smjernice za prikupljanje uzoraka. Web-stranica https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf i Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Web-stranica https:// www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html) i smjernicu Američkog društva za zarazne bolesti (IDSA) (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. Priprema uzoraka i ekstrakcija RNK

Prikupljanje uzorka obavite u skladu s preporukama iz uputa za upotrebu kompleta za ekstrakciju koji koristite, BD MAX™ ExK™ TNA-3. Moguće je da će neki drugi uzorci zahtijevati predobradu. Korisnik treba razviti i validirati postupke pripreme za ekstrakciju specifične za predmetnu primjenu.

Kada se koriste uzorci nazofaringeala ili orofaringeala:

1. Pipetom prenesite između 400 µl i 750 µl nazofaringealnog/orofaringealnog brisa prikupljenog u virusnom transportnom mediju (VTM) ili u BD™ Universal Viral Transport (UVT) System medija u BD MAX™ TNA-3 Sample Buffer Tube epruvetu za uzorak s puferom te je zatvorite čepom s membranom. Izmiješajte uzorak vrtloženjem pri visokoj brzini tijekom 1 minute. Pređite na rad sa BD MAX™ System Operation.

U slučaju korištenja uzoraka sline prikupljenih u transportnom mediju:

1. Uzorci sline mogu se prikupiti u virusnom transportnom mediju (VTM), BD™ Universal Viral Transport (UVT) univerzalnom virusnom transportu, ili IMPROVIRAL™ Viral Preservative Medium (VPM) virusnom konzervirajućem mediju u omjeru 1:3 (slina:medij). Izmiješajte vrtloženjem tijekom 1 minute pri visokoj brzini. Pipetom prenesite 750 µl u BD MAX™ TNA-3 Sample Buffer Tube epruvetu za uzorak s puferom te je zatvorite čepom s membranom. Izmiješajte uzorak vrtloženjem pri visokoj brzini tijekom 1 minute. Pređite na rad sa BD MAX™ System Operation.

U slučaju korištenja čistih uzoraka sline:

1. Kombinirajte slinu s virusnim transportnim medijem (VTM), BD™ Universal Viral Transport (UVT) univerzalnim virusnim transportom, ili IMPROVIRAL™ Viral Preservative Medium virusnim konzervirajućim medijem (VPM) tako da je završni omjer sline:medij 1:3. Izmiješajte vrtloženjem tijekom 1 minute pri visokoj brzini. Zatim pipetom prenesite 750 µl u BD MAX™ TNA-3 Sample Buffer Tube epruvetu za uzorak s puferom te je zatvorite čepom s membranom. Izmiješajte uzorak vrtloženjem pri visokoj brzini tijekom 1 minute. Pređite na rad sa BD MAX™ System Operation.

8.3. Protokol za PCR

Napomena: Detaljne upute potražite u Korisničkom priručniku za BD MAX™ sustav.

8.3.1. Kreiranje programa za testiranje VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System

Napomena: Ako ste već kreirali VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection test, možete preskočiti korak 8.3.1 i preći izravno na 8.3.2.

1) Na zaslonu „Run“ (Pokreni) na BD MAX™ System odaberite karticu „Test Editor“ (Uređivač testa).
2) Kliknite na gumb „Create“ (Kreiraj).
3) Na kartici Basic Information (Osnovne informacije) unuta prozora "Test Name" (Naziv testa), imenujte svoj test: tj. VIASURE *SARS-CoV-2 (N1 + N2)*.
4) S padajućeg izbornika „Extraction Type“ (Vrsta ekstrakcije) odaberite „ExK TNA-3“.
5) S padajućeg izbornika „Master Mix Format“ (Oblik glavne smjese) odaberite „Type 5“ (Tip 5).
    a. Napomena: Proizvod se može koristiti u kombinaciji s dodatnim kompletom VIASURE za BD MAX™ test, a u tom slučaju odaberite opciju „Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)“.
6) U „Sample extraction parameters“ (Parametri ekstrakcije uzorka) odaberite „User defined“ (Korisnički definirani) i prilagodite volumen uzorka na 950 µl.
7) U „Ct Calculation“ (Ct izračun) odaberite „Call Ct at Threshold Crossing“ (Izračunaj Ct pri prelasku granice).
8) Na radnom softveru verzije 5.00 ili više te sa snap-in epruvetama s crtičnim kodom, na izborniku "Custom Barcodes" (Zadani crtični kodovi) odaberite sljedeću konfiguraciju:
    a. Snap-In 2 crtični kod: 1G (u vezi reakcijske epruvete *SARS-CoV-2 (N1 + N2)* reaction tube).
    b. Snap-In 3 crtični kod: 11 (u vezi epruvete s Rehydration Buffer tube).
    c. Snap-In 4 crtični kod: druga reakcijska epruveta VIASURE (drugačija folija) ako odaberete format “Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)” (Dvostruka glavna smjesa koncentriran liofiliziran MM s rehidracijskim puferom - Tip 5) (Odjeljak 8.3.1).
9) Na kartici „PCR settings“ (Postavke za PCR) unesite sljedeće parametre: „Channel Settings“ (Postavke kanala), „Gains“ (Dobit) i „Threshold“ (Granica) (Tablica 3).
    a. Napomena: Proizvod se može koristiti u kombinaciji s dodatnim kompletom VIASURE za BD MAX test, a u tom slučaju „PCR Settings“ (Postavke za PCR) i „Test Steps“ (Koraci testa) treba popuniti za položaje Snap-In 2 (zeleni) i Snap-In 4 (plavi).

Channel (Kanal)	*Alias* (Drugi nazivi)	*Gain* (Dobit)	*Threshold* (Prag)	*Ct Min* (Ct Min)	*Ct Max* (Ct Maks)
475/520 (FAM)	SARS-CoV-2 N2 cilj	80	150	0	40
530/565 (HEX)	Endogeni IC	80	150	0	35
585/630 (ROX)	-	0	0	0	0
630/665 (Cy5)	SARS-CoV-2 N1 cilj	80	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Tablica 3. PCR settings (Postavke za PCR).

Napomena: Preporučuje se postavljanje gore navedenih minimalnih graničnih vrijednosti za svaki kanal kao početnu točku, ali završne postavke mora odrediti krajnji korisnik tijekom tumačenja rezultata kako bi se osiguralo da granice spadaju u eksponencijalnu fazu krivulja fluorescencije i iznad bilo kojeg pozadinskog signala. Granična vrijednost različitih instrumenata može se razlikovati zbog različitih intenziteta signala.

10) Na kartici „PCR settings" (Postavke za PCR) unesite i sljedeće parametre: „Spectral Cross Talk" (Tablica 4).

		***False Receiving Channel* (Kanal s lažnim rezultatima)**				
	***Channel* (Kanal)**	475/520	530/565	585/630	630/665	680/715
***Excitation Channel* (Kanal za pobuđivanje)**	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	-

Tablica 4. Parametri spektralnog preklapanja signala.

11) Na kartici „Test Steps" (Koraci testa) unesite protokol za PCR (Tablica 5).

***Step Name* (Naziv koraka)**	***Profile Type* (Vrsta profila)**	***Cycles* (Ciklusi)**	***Time (s)* (Vrijeme(Vremena))**	***Temperature* (Temperatura)**	***Detect* (Detekcija)**
Reverse transcription (Reverzna transkripcija)	Čekanje	1	900	45°C	-
Initial denaturation (Početna denaturacija)	Čekanje	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection) (Denaturacija i sparivanje/produljenje (prikupljanje podataka)	2-Temperatura	45	10	95°C	-
			61.1	63°C	✓

Tablica 5. Protokol za PCR.

12) Kliknite na gumb „Save Test" (Spremi test).

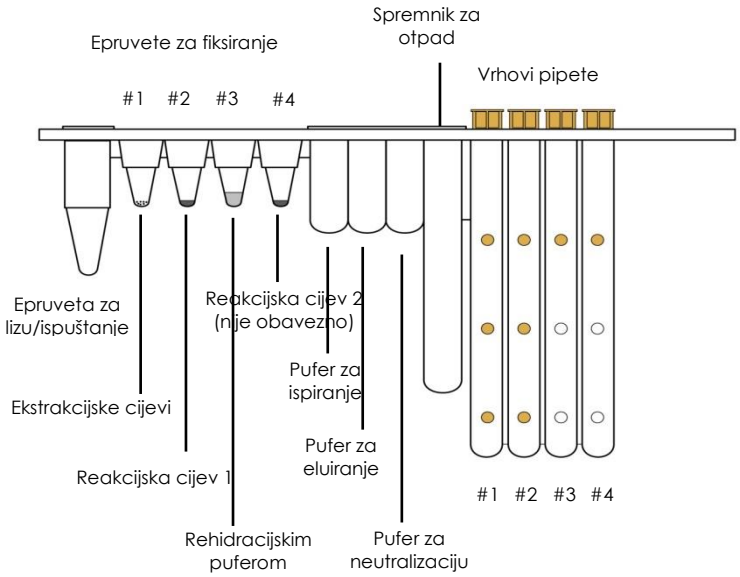
8.3.2. Postavljanje BD MAX™ stalka

1) Za svaki uzorak koji treba testirati izvadite pojedinačne trake s reagensima u bloku iz kompleta (BD MAX™ ExK™ TNA-3 kit). Lagano udarite svaku traku na čvrstu površinu kako biste osigurali da se sve tekućine nalaze na dnu epruveta i stavite ih na stalke za uzorke BD MAX™ sustava.

2) Izvadite potrebni broj BD MAX™ ExK™ TNA Ekstrakcijske cijevi (BD MAX™ ExK™ TNA Extraction Tubes (B4) (bijela folija)) iz njihove zaštitne vrećice. Postavite epruvetu/e za ekstrakciju (bijela folija) u njihove odgovarajuće položaje u TNA traku (položaj 1, bijela boja kodiranje na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite vrećicu patentnom zatvaračem.
3) Odredite i razdvojite odgovarajući broj *SARS-CoV-2 (N1 + N2)* reaction tubes (1G folija) i postavite ih u njihove odgovarajuće položaje na traci (Snap položaj 2, označen zelenom bojom na stalku). Pogledajte Sliku 1).

    a. Istisnite višak zraka i zatvorite aluminijske vrećice patentnim zatvaračem.
    b. U cilju obavljanja pravilnog postupka rehidracije pazite da se liofilizirani proizvod nalazi na dnu epruvete te da ne prianja za gornji dio epruvete ili za zatvarač od folije. Lagano pritisnite svaku epruvetu na tvrdoj površini kako biste se uvjerili da je sav proizvod na dnu epruvete.
        i. Napomena: Ako odaberete oblik „Dual Master Mix Concentrated Lyophilized MM with rehydration Buffer (Type 5)“ (Dvostruka glavna smjesa koncentriran liofiliziran MM s rehidracijskim puferom - Tip 5) (odjeljak 8.3.1), odredite i razdvojite odgovarajući broj dodatnih VIASURE reakcijskih epruveta (drugačija folija) i postavite ih u njihove odgovarajuće položaje na traci (položaj 4, označen plavom bojom na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite aluminijske vrećice patentnim zatvaračem.
4) Izvadite odgovarajući broj epruveta Rehydration Buffer tubes (11 folija) i postavite ih u njihove odgovarajuće položaje na traci (položaj 3, nije obojen na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite vrećicu patentnim zatvaračem.
    a. U cilju osiguravanja pravilnog prijenosa pazite da se tekućina nalazi na dnu epruvete te da ne prianja za gornji dio epruvete ili za zatvarač od folije. Lagano pritisnite svaku epruvetu na tvrdoj površini kako biste se uvjerili da je sav proizvod na dnu epruvete.

Slika 1. BD MAX™ TNA traka s reagensima (TNA) iz kompleta BD MAX™ ExK™ TNA-3 kit.



8.3.3. Postavljanje BD MAX™ instrumenta

1) Odaberite karticu „Work List“ (Radni popis) na zaslonu „Run“ (Pokreni) na BD MAX™ sustavu s verzijom softvera v4.50A ili novijom.
2) S padajućeg izbornika „Test“ (Test) odaberite VIASURE *SARS-CoV-2 (N1 + N2)* (ako test nije već kreiran, pogledajte odjeljak 8.3.1).
3) Odaberite odgovarajući broj serije kompleta (naveden na vanjskoj kutiji kompleta za ekstrakciju kojeg koristite) s padajućeg izbornika (neobavezno).
4) Unesite identifikacijski broj „Sample Buffer tube“ (epruvete za uzorak s puferom) na kartici „Worklist“ (Radni popis) skeniranjem crtičnog koda pomoću sekenera ili ručnim unošenjem broja.
5) Popunite polje „Specimen/Patient ID“ (ID uzorka/bolesnika) i/ili prozor „Accession“ (Pristup) na kartici „Worklist“ (Radni popis) i kliknite na gumb „Save“ (Spremi). Nastavite s postupkom dok ne unesete sve epruvete za uzorak s puferom. Pazite da se ID uzorka/bolesnika i epruvete za uzorak s puferom podudaraju.
6) Stavite pripremljenu epruvetu za uzorak s puferom na BD MAX™ stalak/e.
7) Stavite stalak/e u BD MAX™ System (stalak A se nalazi lijevo u odnosu na BD MAX™ System, dok se stalak B nalazi na desnoj strani).
8) Stavite potrebni broj uložaka BD MAX™ PCR Cartridge(s) u BD MAX™ System.
9) Zatvorite vrata BD MAX™ System.
10) Kliknite „Start Run“ (Pokreni postupak) da započnete postupak.

8.3.4. BD MAX™ izvješće

1) U glavnom izborniku kliknite na gumb „Results“ (Rezultati).
2) Kliknite dvaput na svoj postupak na popisu ili pritisnite gumb „view“ (prikaz).
3) Kliknite na „Print“ (Ispiši) i odaberite: “Run Details, Test Details and Plot…” (Podaci o postupku, podaci o testu i grafikon...“.
4) Kliknite na gumb „Print or Export“ (ispiši ili izvezi) na zaslonu „Run Reports“ (Izvješća o postupku).

9. Tumačenje rezultata

Detaljne upute za analiziranje podataka potražite u Korisničkom priručniku za BD MAX™ sustav.

Analizu podataka obavlja BD MAX™ softver prema uputama proizvođača. BD MAX™ softver kreira izvješće o Ct vrijednostima i amplifikacijskim krivuljama za svaki kanal detekcije svakog uzorka testiranog na sljedeći način:

- Ct vrijednost 0 znači da softver nije izračunao Ct vrijednost s navedenom granicom (pogledajte Tablicu 3). Amplifikacijska krivulja uzorka koja pokazuje „0“ za Ct vrijednost mora se provjeriti ručno.

- Ct vrijednost -1 znači da nije došlo do amplifikacijskog procesa.

- Svaku drugu Ct vrijednost treba tumačiti u vezi s amplifikacijskom krivuljom i prema smjernicama za tumačenje uzoraka navedenim u Tablici 6.

Provjerite unutarnji kontrolni signal da potvrdite funkcionira li amplifikacijska smjesa ispravno. Pored toga, provjerite da nema izvješća o kvaru BD MAX™ sustava.

Pomoću sljedeće tablice očitajte i analizirajte rezultate:

SARS-CoV-2 (N2 cilj) (475/520)	Endogena unutarnja kontrola (530/565)	SARS-CoV-2 (N1 cilj) (630/665)	Tumačenje
+	+/-[1]	+	**SARS-CoV-2 *N genska* RNK detektirana [1]**
+[2]	+/-[1]	-	**SARS-CoV-2 *N genska* RNK detektirana [1,2]**
-	+/-[1]	+[2]	**SARS-CoV-2 *N genska* RNK detektirana [1,2]**
-	+[3]	-	**SARS-CoV-2 *N* genska RNK nije otkrivena[3]**
-	-[3]	-	**Resultado no resuelto (UNR) debido a la presencia de inhibidores en la reacción de PCR o a un problema general (no informado por un código de error) durante el procesamiento de la muestra y/o la etapa de amplificación[3]**
IND	IND	IND	**Rezultat testa nije moguće utvrditi (IND). Zbog kvara BD MAX™ System. Rezultat testa prikazan u slučaju kvara instrumenta povezanog sa šifrom o pogrešci.**
INC	INC	INC	**Nepotpun rezultat testa (INC). Zbog kvara BD MAX™ System. Rezultat testa prikazan u slučaju neuspješnog obavljanja postupka.**

Tablica 6. Tumačenje rezultata

+: Došlo je do amplifikacije

-: Nije došlo do amplifikacije

1 Uzorak se smatra pozitivnim ako je dobivena Ct vrijednost manja od 40. Endogena interna kontrola (IC) može ili ne mora pokazivati signal amplifikacije. Ponekad detekcije interne kontrole nije potrebna jer veliki broj kopija cilja može uzrokovati preferencijalnu amplifikaciju ciljno-specifičnih nukleinskih kiselina.

2 Ako se samo jedno ciljno mjesto *N* gena amplificira, potvrdite sigmoidni oblik krivulje te intenzitet fluorescencije. U slučaju sumnjivog tumačenja, ovisno o dostupnom materijalu, također se preporučuje:

a) ponovno ekstrahiranje i testiranje drugog alikvota istog uzorka (ako je moguće povećajte volumen uzorka na 750 µl) ili

b) uzimanje novog uzorka te ponovno testiranje.

3 U slučaju negativnih ciljnih mjesta SARS-CoV-2, unutarnja kontrola mora pokazati signal amplifikacije s Ct manjim od 35. Ct vrijednosti mogla bi varirati zbog endogene interne kontrole te se radi o domaćinskom genu koji bi morao biti prisutan u svim ljudskom stanicama s jezgrom u izvornom uzorku. Ako postoji odsutno signala ili Ct vrijednost ≥ 35 endogene interne kontrole, rezultat se smatra "Neriješenim" te je potrebno ponovno testiranje.

U slučaju stalnog dvosmislenog rezultata preporučuje se pregledati upute za uporabu te proces ekstrakcije kojeg koristi korisnik; za potvdu ispravne učinkovitosti svakog RT-qPCR koraka te pregled parametara; te za provjeru sigmoidnog oblika krivulje i intenzitet fluoresencije.

Rezultate testa treba procijeniti zdravstveni djelatnik uzimajući u obzir anamnezu, kliničke simptome i druge dijagnostičke testove.

10. Ograničenja testa

- Rezultate testa treba procijeniti zdravstveni djelatnik uzimajući u obzir anamnezu, kliničke simptome i druge dijagnostičke testove.
- Iako se ovaj test može koristiti s drugim vrstama uzoraka, validiran je s nazofaringealni/orofaringealni brisevi i uzorci sline prikupljenima u VTM.
- Za dobru učinkovitost testa liofilizirani proizvod mora biti na dnu epruvete te ne smije prianjati na gornjem dijeli epruvete ili čepa od folije. Lagano pritisnite svaku epruvetu na tvrdoj površini kako biste se uvjerili da je sav proizvod na dnu epruvete.
- Izgled reakcije smjese u stabiliziranom formatu koji se obično nalazi na dnu epruvete razlikuje se od uobičajenog (bez stožastog oblika, inhomogeni, manji/veći i/ili bojom različit od bjeličastog) ne mijenja funkcionalnost testa.
- Kvaliteta testa ovisi o kvaliteti uzorka; mora se ekstrahirati odgovarajuća nukleinska kiselina iz respiratornih uzoraka.
- Ovaj test je samo kvalitativne test te ne osigurava kvantitativni vrijednosti te ne ukazuje na broj prisutnih organizama.
- Izuzetno niske razine ciljnog elementa ispod razine detekcije mogu biti detektirane, no moguće je da se rezultati neće moći ponoviti.
- Postoji mogućnost pojave lažnih pozitivnih rezultata zbog unakrsne kontaminacije izazvane uzorcima suspektnima na SARS-CoV-2 koji sadrže visoke koncentracije ciljne RNK ili kontaminacije izazvane proizvodima koji sadrže PCR iz ranijih reakcija.
- Kombinacije specifične početnice i sonde za detekciju uščuvanih regija *N* gena koji se koristi u VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System osmišljeni su na temelju američkog Centra za kontrolu bolesti za specifičnu detekciju SARS-CoV-2 amplifikacijom dvije jedinstvene regije *N* gena. Ne pokazuju značajnu kombiniranu homologiju s ljudskim genomom, ljudskom mikroflorom, SARS-CoV ili ostalim koronavirusima što bi moglo rezultirati u predvidivim lažno pozitivnim rezultatima.
- Lažno negativni rezultati mogu nastati uslijed nekoliko čimbneika te njihovih kombinacija uključujući:
    - nepravilno prikupljanje uzoraka, transport, pohrana i/ili metode rukovanja.
    - Nepravilne postupke obrade (uključujući ekstrakciju RNK).
    - Degradacija virusne RNK tijeko otpreme/pohrane i/ili obrade uzorka.
    - Mutacije ili polimorfizmi na veznim regijama početnice ili sonde mogu utjecati na detekciju novih ili nepoznatih varijanti SARS-CoV-2.
    - Virusno opterećenje u uzorku koje je ispod granice detekcije za test.
    - Prisutnost RT-qPCR inhibitora ili drugih tipova interferirajućih tvari. Nisu procijenjeni utjecaji cjepiva, antivirusnih terapija, antibiotika, kemoterapijskih sredstva ili imunosupresiva korištenih za prevenciju COVID-19 ili tijekom liječenja infekcije.
    - Neuspjeh u pridržavanju uputa za uporabu te prilikom postupka testiranja.
- Amplifikacija pojedinačnog ciljnog mjesta ili čak slučajno pozitivnih rezultata ukazuje na blago različito iskorištenje amplifikacije ciljnog mjesta *N* gena. Uzorci s niskim virusnim opterećenjem mogu rezultirati u *N* pojedinačnoj ciljnoj amplifikaciji. U slučaju sumnji, preporučuje se obratiti se referentnom laboratoriju za daljnje testiranje, ako je klinički indicirano.

- Neki uzorci možda neće iskazati amplifikacijske krivulje *RNase P* zbog niskog broja ljudskih stanica u izvornom kliničkom uzorku. Negativni signal unutarnje kontrole ne isključuje prisutnost RNK virusa SARS-CoV-2 u kliničkom uzorku.
- Pozitivan rezultat testa ne ukazuje nužno na prisutnost vidljivih virusa te ne ukazuje da su ti virusi infektivni ili uzročni agensi za kliničke simptome. Međutim, pozitivni rezultat ukazuje na prisutnost ciljnih virusnih sekvenci (*N* gena).
- Negativni rezultati ne isključuju infekciju SARS-CoV-2 i ne smiju se koristiti kao jedini temelj za odlučivanje o liječenju ili pružanju druge zdravstvene skrbi bolesniku. Nisu određene optimalne vrste uzoraka i izračun vremena vršnih razina virusa tijekom infekcija čiji je uzrok SARS-CoV-2. Za detekciju virusa može biti potrebno prikupljanje više uzoraka (vrste i vremenske točke) od istog bolesnika.
- Ako su dijagnostički testovi za ostale respiratorne bolesti negativni, a klinička slika bolesnika te epidemiološke informacije sugeriraju da je moguća infekcija SARS-CoV-2, potrebno je razmotriti mogućnost lažnog negativnog rezultata i ponovnog testiranja bolesnika.
- U slučaju neriješenih, neutvrdivih ili nepotpunih rezultata primjenom kompleta za detekciju VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System potrebno je ponovno testiranje. Neriješeni rezultati mogu biti posljedica prisutnosti inhibitora u uzorku ili nepravilne rehidracije epruvete s liofiliziranom reakcijskom smjesom. Ako postoji kvar na instrumentu, dobivaju se neutvrdivi ili nepotpuni rezultati.

11. Kontrola kvalitete

Komplet VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System sadrži endogenu unutarnju kontrolu (IC) u svakoj reakcijskoj epruveti pomoću koje se potvrđuje pravilno provođenje tehnike.

12. Radne karakteristike

12.1. Klinička osjetljivost i specifičnost

Klinička učinkovitost kompleta VIASURE *SARS-CoV-2 (N1+N2)* Real Time PCR Detection Kit for BD MAX™ System testirana je na nazofaringealnim brisevima i orofaringealnim brisevima bolesnika sa sumnjom na respiratornu infekciju. Dobiveni su sljedeći rezultati:

	Mjesto	Vrsta uzorka	Radni proces	Cilj
1	Hospital Universitario Miguel Servet (HUMS)	nazofaringealnim brisevima	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	SARS-CoV-2
2	"Servicio de Microbiología" Hospital Universitario Marqués de Valdecilla (Santander, Spain)	nazofaringealnim brisevima	MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit pomoću KingFisher Flex System instrument (ThermoFisher) + BD MAX™ System	SARS-CoV-2

Tablica 7. Mjesto, vrsta uzorka, radni proces i cilj.

Istinski pozitivne i negativne vrijednosti, lažne pozitivne i negativne vrijednosti, vrijednosti osjetljivosti i specifičnosti za komplet VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System izračunate su u odnosu na svaki komparativni test kako je prikazano u sljedećim tablicama:

Mjesto	Komparativni test	Target	TP	TN	FP	FN	Osjetljivosti	Specifičnosti
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*

Tablica 8. Istinski pozitivne i negativne vrijednosti, lažne pozitivne i negativne vrijednosti, osjetljivosti i specifičnosti vrijednosti za komplet VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

Izračun specifičnosti testa nije se mogao izvršiti zbog činjenice da negativni uzorci nisu analizirani.

Kako bi se procijenila kompatibilnost različitih matrica uzoraka (nazofaringealni bris, orofaringealni bris i nazofaringealni/orofaringealni bris u VTM-u tvrtke Vircell), provedena je studija kompatibilnosti. Dobiveni rezultati pokazali su da su tri različite matrice uzoraka kompatibilne s reakcijskom epruvetom *SARS-CoV-2 (N1 + N2)* reaction tube.

Procijenjena je učinkovitost kompleta VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System sustav s uzorcima sline. Testirani su negativni pojedinačni uzorci sline u koje je dodana poznata koncentracija smrznute kvantificirane kulture novog koronavirusa 2019, soj:2019-nCoV/USA-WA-1/2020 (ATCC-VR-1986HK) inaktivirane toplinom. Zamišljeno je da se procjena provede sa 30 pozitivnih uzoraka (20 uzoraka 2 puta LoD (2xLoD), što odgovara 0,53 kopije genoma (GC)/µL i 10 uzoraka 5 puta LoD (5xLoD), što odgovara 1,32 kopija genoma (GC)/µL) te 10 negativnih uzoraka. Ovaj je test izveden korištenjem volumena uzorka od 750 µl od svakog uvjeta dodanog u epruvetu za uzorak s puferom (Sample Buffer Tube) (SBT) kompleta za ekstrakciju TNA-3 (TNA-3 Extraction Kit) i pokrenut u cjelovitom načinu rada (automatizirana ekstrakcija i PCR amplifikacija) korištenjem BD MAX™ ExK™ TNA-3.

Postotak slaganja izračunat je u odnosu na očekivani rezultat za svaki pojedini uzorak, a rezultati su prikazani u sljedećoj tablici.

Uzorak sline	Slaganje
Pozitivni uzorak (2xLoD)	97.5%
Pozitivni uzorak (5xLoD)	100%
Negativni uzorak	100%

Tablica 9. Istinski pozitivne i negativne vrijednosti, lažne pozitivne i negativne vrijednosti, osjetljivosti i specifičnosti vrijednosti za komplet VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

Zaključno, uzorci sline bili su kompatibilni s kompletom VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

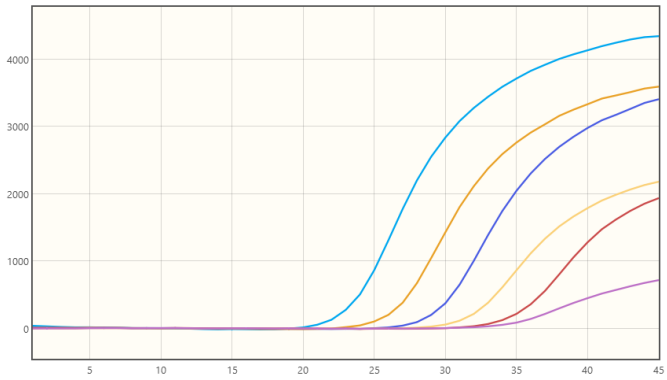
Rezultati pokazuju veliko slaganje za detekciju SARS-CoV-2 uporabom VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analitička osjetljivost

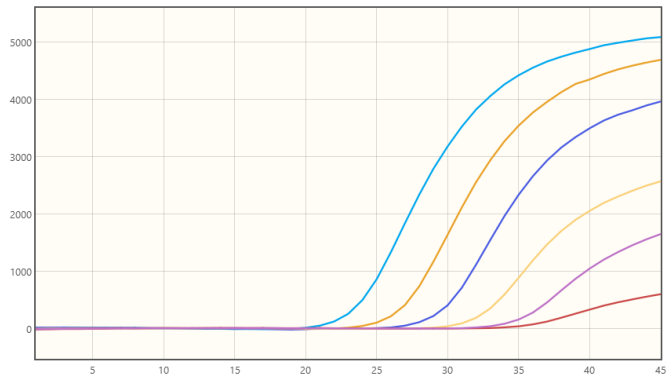
Granica detekcije kompleta VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System sustav iznosi ≥5 kopija genoma po reakciji na nazofaringealnim brisevima i ≥10 kopija genoma po reakciji na uzorcima sline s pozitivnom stopom od ≥95%.

Napomena: Granica detekcije na uzorcima sline izračunata je korištenjem volumena uzorka od 750 µL (razrjeđivanje 1:3 u VTM-u).

Slika 2. Serije razrjeđivanja predloška SARS-CoV-2 *(N1 + N2)* ($9.9*10^4$-$9.9*10^0$ te $5.0*10^0$ kopija genoma po reakciji) na sustavu BD MAX™ System (475/520 (FAM) kanal).



Slika 3. Serija razrjeđivanja predloška SARS-CoV-2 *(N1 + N2)* ($9,9*10^4$-$9,9*10^0$ te $5,0*10^0$ kopija genoma po reakciji) radi na sustavu BD MAX™ System (630/665 (Cy5) kanal).



12.3. Analitička specifičnost

Specifičnost testa SARS-CoV-2 *(N1 + N2)* potvrđena je testiranjem panela koji se sastoji od različitih mikroorganizama koji predstavljaju najčešće respiratorne patogene. Nije zabilježena unakrsna reaktivnost između bilo kojih od sljedećih testiranih mikroorganizama:

Testiranje unakrsne reaktivnosti					
Ljudski adenovirus tipovi 1-5, 8, 15, 31, 40 i 41	-	Influenza A/New Caledonia/20/99(H1N1) virus	-	*Legionella longbeachae*	-
Ljudski bokavirus	-	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	-	Ljudski metapneumovirus A i 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* koja nije otporna na rifampicin	-
Chlamydia psittaci genotip A i C	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-	Virusi ljudske parainfluence tipa 1, 2, 3 i 4	-
Chlamydophila pneumoniae CM-1	-	Influenza A/ South Australia/55/2014, IVR-175 (H3N2) virus	-	*Pneumocytis jirovecii* tip A1 i g885652	-
Ljudski koronavirus 229E, OC43, NL63 i HKU1	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus	-	Ljudski rinovirus tip C	-
MERS koronavirus	-	Influenza A/Anhui/1/2013 (H7N9) virus	-	*Staphylococcus aureus* podsoj *aureus*	-
SARS koronavirus soj Frankfurt 1	-	Influenza B/Brisbane/60/2008 virus	-	*Staphylococcus epidermidis*	-
Enterovirus 68 i 71	-	Influenza B/Florida/04/06 virus	-	*Streptococcus pneumoniae Z022*	-
Enterovirus Echovirus 11 i 30	-	Influenza B/Phuket/3073/2013 virus	-	*Streptococcus pyogenes*	-
Enterovirus Coxsackievirus A24, A9 i B3	-	*Legionella bozemanii*	-	*Streptococcus salivarius*	-
Haemophilus influenzae MinnA	-	*Legionella dumoffii*	-	Respiratorni sincicijski virus (RSV) A i B	-

Tablica 10. Referentni patogeni mikroorganizmi korišteni u ovom ispitivanju.

12.4. Analitička reaktivnost

Reaktivnost VIASURE *SARS-CoV-2 (N1 + N2)* Real Time PCR Detection Kit for BD MAX™ System za SARS-CoV-2 procijenjena je naspram RNK iz ljudskog soja 2019-nCoV BetaCoV/Germany/BavPat1/2020 p.1, ljudski soj 2019-nCoV 2019-nCoVg/Italy-INMI1, SARS-CoV-2 soj 2019nCoV/USA-WA1/2020, SARS-CoV-2 soj BetaCoV/Berlin/ChVir1670/2020_IsolatBER, SARS-CoV-2 soj BetaCoV/Munich/ChVir984/2020, SARS-CoV-2 soj BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER sintetska RNK kontrolira četiri varijante virusa SARS-CoV-2: SARS-CoV-2 izolat Australia/VIC01/2020, SARS-CoV-2 izolat Wuhan-Hu-1, B.1.1.7_710528 i B.1.1.7_601443, i pokazala je pozitivne rezultate.

Bibliography/Literatura

1. Huang, C. *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, 2020. DOI : 10.1016/S0140-6736(20)30183-5.
2. Zhu N. *et al*. A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*., 2020. DOI : 10.1056/NEJMoa2001017.
3. World Health Organization. MERS situation update. January 2020. Available from https://applications.emro.who.int/docs/EMCSR254E.pdf?ua=1 Accessed January 2021.
4. Chen N. *et al.*. Epidemiological and Clinical Characteristics of 99 Cases of 2019-Novel Coronavirus (2019-nCoV) Pneumonia in Wuhan, China. *The Lancet*, 2020. DOI: 10.1016/S0140-6736(20)30211-7.
5. Lv D.F. *et al*. Dynamic change process of target genes by RT-PCR testing of SARS-Cov-2 during the course of a Coronavirus Disease 2019 patient. *Clinica Chimica Acta* 2020; 506: 172-175.
6. World Health Organization. Clinical management of COVID-19 disease" Interim guidance 27 May 2020. Available from https://www.who.int/publications/i/item/clinical-management-of-covid-19 Accessed January 2021.
7. Lu R. *et al*. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet,* 2020. DOI : 10.1016/S0140-6736(20)30251-8.
8. Rothe C. *et al*. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. *New England Journal of Medicine*, 2020. DOI : 10.1056/NEJMc2001468.
9. Centers of Disease Control and Prevention (CDC). Coronavirus Disease 2019 (COVID-19), Symptoms of Coronavirus. Available from https://www.cdc.gov/coronavirus/2019-ncov/about/symptoms.html Accessed January 2021.
10. Centers of Disease Control and Prevention (CDC). Coronavirus Disease 2019 (COVID-19), Older Adults. Available from https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/older-adults.html Accessed January 2021.
11. World Health Organization. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. Interim guidance. 19 March 2020. Available from https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 Accessed January 2021.
12. Yan Y *et al.* Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): Current status, challenges, and countermeasures. *Reviews in Medical Virology* 2020; 30(3):e2106.
13. Centers of Disease Control and Prevention (CDC). 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes. https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf Accessed January 2021.
14. Chu D.K.W. *et al*. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. *Clinical Chemistry* 2020;66(4): 549-555.
15. Corman V.M. *et al*. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *European communicable disease bulletin* 2020;25(3).
16. European Centre for Disease Prevention and Control. Novel coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK – sixth update – 12 March 2020. Stockholm: ECDC; 2020. Available from https://www.ecdc.europa.eu/sites/default/files/documents/RRA-sixth-update-Outbreak-of-novel-coronavirus-disease-2019-COVID-19.pdf Accessed January 2021.
17. Lim, Y. X., Ng, Y. L., Tam, J. P., & Liu, D. X. (2016). Human coronaviruses: a review of virus–host interactions. *Diseases*, 4(3), 26.

18. McBride R. *et al.* The coronavirus nucleocapsid is a multifunctional protein. *Viruses* 2014; 6(8):2991-3018.
19. Sheikh A. *et al*. Analysis of preferred codon usage in the coronavirus *N* genes and their implications for genome evolution and vaccine design. *Journal of Virological Methods* 2020; 277:113806.
20. World Health Organization. Laboratory testing strategy recommendations for COVID-19: interim guidance Interim guidance. 21 March 2020. Available from https://www.who.int/publications/i/item/laboratory-testing-strategy-recommendations-for-covid-19-interim-guidance Accessed January 2021.

Symbols for IVD components and reagents/ Simboli za IVD komponente i reagense

IVD	*In vitro* diagnostic device *In vitro* dijagnostički uređaj		Keep dry Čuvati na suhom		Use by Rok valjanosti		Manufacturer Proizvođač
LOT	Batch code (Lot) Šifra serije		Consult instructions for use Pogledajte upute za upotrebu		Temperature limitation Ograničenje temperature	Σ	Contains sufficient for <n> test Sadržaj dovoljan za <n> test(ova)
DIL	Sample diluent Razrjeđivač uzorka	REF	Catalognumber Kataloški broj				

Trademarks

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

Change Control / Kontrola promjene		
Version No. / Verzija br.	**Changes / Promjene**	**Date / Datum**
00	Original version/ Izvorna verzija.	21/05/2021

Table A 2. Control change table / Tablica kontrole promjene.

Revision: 21st May 2021

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F-566 rev01