VIASURE

Real Time PCR Detection Kits

by CerTest BIOTEC

SARS-CoV-2, Flu (A+B) & RSV

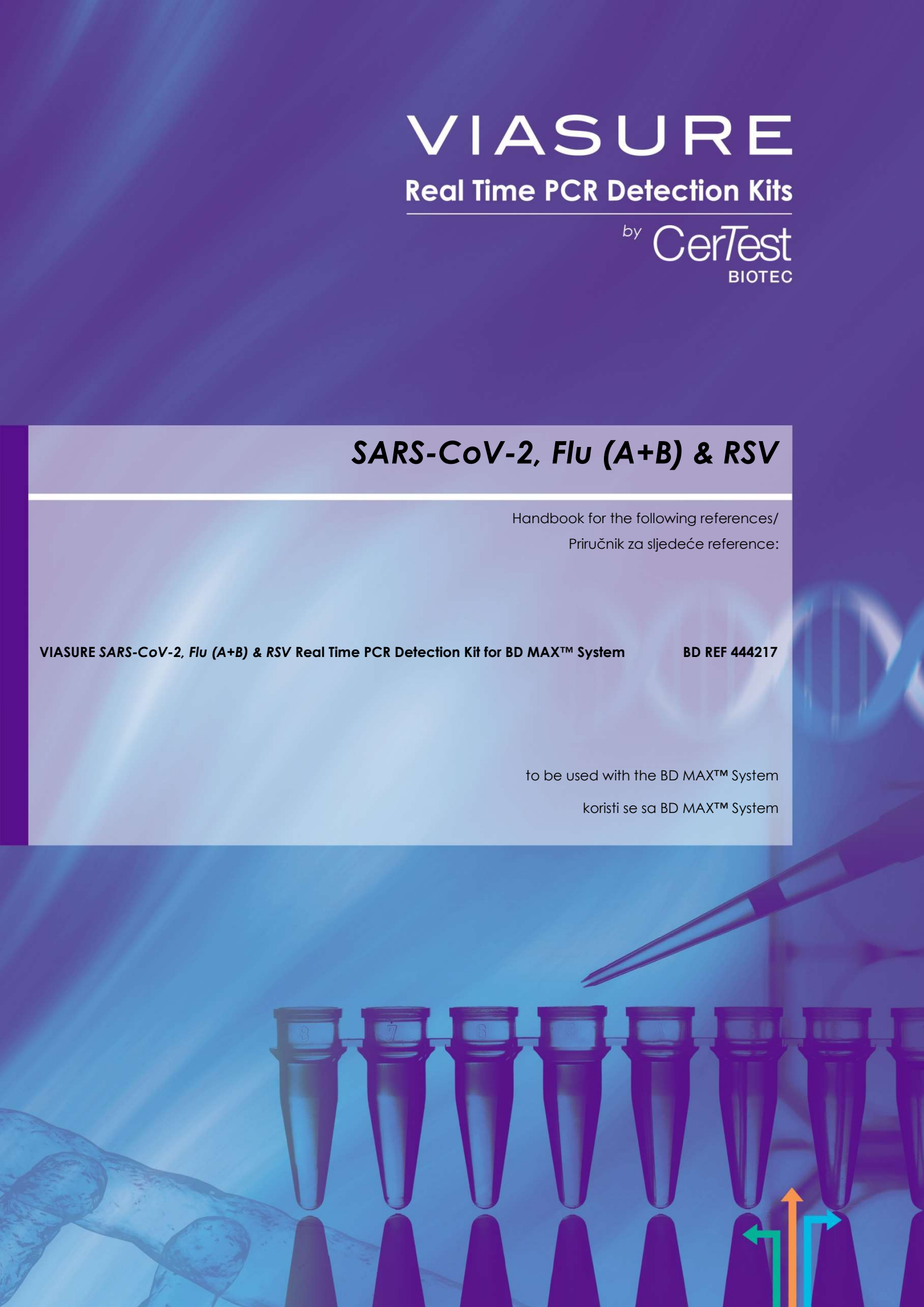
Handbook for the following references/

Priručnik za sljedeće reference:

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System **BD REF 444217**

to be used with the BD MAX™ System

koristi se sa BD MAX™ System



ENGLISH

1. Intended use

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System is an automated real-time RT-PCR test designed for the qualitative detection and differentiation of RNA from the SARS-CoV-2, Influenza A (Flu A), Influenza B (Flu B) and/or Human Respiratory Syncytial Virus A/B (RSV) in respiratory samples from individuals suspected of COVID-19 or other respiratory infection by their healthcare provider. This test is intended to be used as an aid in the identification of the presence of the SARS-CoV-2, Flu A, Flu B and/or RSV viral RNA. 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 respiratory specimens, amplified using RT-PCR and detected using fluorescent reporter dye probes specific for SARS-CoV-2, Flu A, Flu B and/or RSV.

2. Summary and Explanation

Coronavirus are enveloped non-segmented positive-sense RNA viruses and belong to *Coronaviridae* family. There are six coronavirus species known to cause human diseases. 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. 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.

In December 2019, some people that worked at or lived around the Huanan seafood market in Wuhan, Hubei Province, China, have presented pneumonia of unknown cause. Deep sequencing analysis of the respiratory samples indicated a novel coronavirus, which was named firstly 2019 novel coronavirus (2019-nCoV) and lately SARS-CoV-2.

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. Although the pneumonia is the principal illness associated, a few patients have developed severe pneumonia, pulmonary edema, acute respiratory distress syndrome, or multiple organ failure and death. Centers of Disease Control and Prevention (CDC) believes that symptoms of SARS-CoV-2 may appear in as few as 2 days or as long as 14 days after exposure, being the most common fever or chills, cough, fatigue, anorexia, myalgia and dyspnea. Less common symptoms are sore throat, nasal congestion, headache, diarrhea, nausea and vomiting. Loss of smell (anosmia) or loss of taste (ageusia) preceding the onset of respiratory symptoms has also been reported. 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.

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) specimens collected mainly by a healthcare provider) and/or lower respiratory specimens (sputum, endotracheal aspirate, or

bronchoalveolar lavage in patients with more severe respiratory disease) for the identification of SARS-CoV-2 and other respiratory viruses, such as Influenza and RSV.

Influenza viruses belong to the *Orthomyxoviridae* family and cause the majority of viral lower respiratory tract infections. Influenza A and B are a significant cause of morbidity and mortality worldwide, considering that elderly and compromised individuals are especially at risk of developing severe illness and complications such as pneumonia. People feel some or all of these symptoms: fever or feeling feverish/chills, cough, sore throat, nasal stuffiness and discharge, myalgia, headaches, and anorexia. The influenza viruses can be spread from person to person in two different ways: through the air (large droplets and aerosols from sneezing and coughing), and by direct or indirect contact.

Influenza A and B are an enveloped, single stranded RNA viruses that containing eight segmented strands of genome RNA, which typically encodes 11 or 12 viral proteins. The viral envelope, derived from the host plasma membrane, consists of a lipid bilayer containing transmembrane proteins, like hemagglutinin (HA) and neuraminidase (NA), and matrix proteins M1 and M2. Influenza A viruses are further classified into subtypes based on the antigenicity of their "HA" and "NA" molecules, whereas Influenza B is divided into 2 antigenically and genetically distinct lineages, Victoria and Yamagata.

Human respiratory syncytial viruses A and B (RSV) belong to the *Paramyxoviridae* family and are the most important viral agents of acute respiratory infections. RSV is an enveloped, nonsegmented, negative, single stranded linear RNA genome virus. Respiratory syncytial virus is a common contributor of respiratory infections causing bronchitis, pneumonia, and chronic obstructive pulmonary infections in people of all ages. People often feel some or all of these symptoms: rhinorrhea, low-grade fever, cough, sore throat, headache, and wheezing. RSV is transmitted via large nasopharyngeal secretion droplets from infected individuals, close contact, or self-inoculation after touching contaminated surfaces.

Diagnosis can be problematic, as a wide range of pathogens can cause acute respiratory infections presenting with similar clinical syndromes. Real-time PCR assays have been shown to be a sensitive and specific diagnostic tool for the detection of SARS-CoV-2, Flu A, Flu B and RSV viruses.

3. Principle of the procedure

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System is designed for the identification of SARS-CoV-2, Flu A, Flu B and /or RSV in respiratory 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) for SARS-CoV-2, a conserved region of the *M1* gene for Flu A and Flu B, and a conserved region of the *N* gene for RSV using specific primers and fluorescent-labeled probes.

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* 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, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System is composed of two different reaction tubes. One of the tubes detects and differentiates the RNA from Flu A, Flu B and/or RSV (Transparent Red or 1A foil) and the other tube detects specifically the RNA from SARS-CoV-2 (Transparent Green or 1G foil). Each tube contains 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 internal control (endogenous in the SARS-CoV-2 reaction tube) to monitor the extraction process and/or inhibition of the polymerase activity. The SARS-CoV-2 assay uses a human housekeeping gene as an endogenous Internal Control (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. Each RNA targets are amplified and detected in specific channels (475/520, 585/630, and/or 630/665) and the internal control (IC) in channel 530/565. In the Flu A, Flu B and/or RSV assay, Flu A RNA target is amplified and detected in channel 475/520, Influenza B RNA target in channel 585/630, RSV RNA target in channel 630/665 and the internal control (IC) of this assay in channel 530/565. In SARS-CoV-2 assay, N2 target is amplified and detected in channel 475/520, N1 target in channel 630/665 and the endogenous internal control (IC) in channel 530/565.

4. Reagents provided

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System includes the following materials and reagents detailed in Table 1:

Reference	Reagent/Material	Description	Color/Barcode	Amount
VS-ABR212R	*Flu A, Flu B & RSV* reaction tube	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers and internal control in stabilized format	Transparent Red or 1A foil	2 pouches of 12 tubes
VS-NCO312	*SARS-CoV-2 (N1 + N2)* reaction tube	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers and endogenous internal control in stabilized format	Transparent Green or 1G foil	2 pouches of 12 tubes
VS-RB09	Rehydration Buffer tube	Solution to reconstitute the stabilized product	Transparent Orange or 11 foil	1 pouch of 24 tubes

Table 1. Reagents and materials provided in VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System with Cat. N°. VS-FNR124 (444217).

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, Flu (A+B) & RSV* 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 indented 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, Flu (A+B) & RSV* 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. Avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents at all times. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges.
- Make sure to use a tube to determine RNA from Influenza A, Influenza B and RSV in Snap-In 2 (green position) and another tube to determine RNA from SARS-CoV-2 in Snap-In 4 (blue position). Be careful not to mix them throughout the entire process.
- 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 or smoke in the working area. Wash your hands after finishing the test.
- Samples must be treated as potentially infectious 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, storage, treatment and disposal of samples.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Procedure

8.1. SAMPLE COLLECTION, STORAGE AND TRANSPORT

The VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System has been validated on nasopharyngeal/ oropharyngeal swab collected in viral transport media (VTM) Vircell S.L., Spain).

Other types of samples from nasopharyngeal/oropharyngeal swabs in VTM must be validated by the user.

Collection, storage and transport specimens should be maintained per the conditions validated by the user. Overall, respiratory samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type) and processed as soon as possible to guarantee the quality of the test. The specimens should be transported at 2 to 8°C for up to 48 hours, following the local and national regulations for the transport of pathogen material. For long term transport (more than 48 hours), we recommend shipping at ≤-20°C. It is recommended to use fresh specimens for the test. The samples can be stored at 2 to 8°C for up to 48 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.

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.

1. Pipette 400 µL of nasopharyngeal/ oropharyngeal swab collected in viral transport media (VTM) into a BD MAX™ 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.

Note: The *Flu A, Flu B & RSV* reaction tube has been validated with a sample volume of 200-400 µL and the *SARS-CoV-2 (N1 + N2)* reaction tube with a sample volume of 400-750 µL.

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, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System

Note: If you have already created the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection test, 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, Flu (A+B) & RSV* (VSARSCoV2,FluA+B,RSV).
4) In the "Extraction Type" drop down menu, select "ExK TNA-3".
5) In the "Master Mix Format" drop down menu, choose "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 and have barcoded foil snap-in tubes, in the "Custom Barcodes" select the following configuration:
    a. Snap-In 2 Barcode: 1A (concerning *Flu A, Flu B & RSV* reaction tube)
    b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube)
    c. Snap-In 4 Barcode: 1G (concerning *SARS-CoV-2 (N1 + N2)* reaction tube)
9) "PCR Settings" and "Test Steps" must be completed for Snap-In 2 (green) and Snap-In 4 (blue) positions.
10) Snap-In 2 (green). In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 2).

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	Flu A	60	100	0	40
530/565 (HEX)	IC	80	300	0	40
585/630 (ROX)	Flu B	60	200	0	40
630/665 (Cy5)	RSV	60	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Table 2. 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.

11) Snap-In 2 (green). In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 3), as well.

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	0.0	0.0	0.0	0.0
	530/565	0.0	-	2.0	0.0	0.0
	585/630	0.0	0.0	-	0.0	0.0
	630/665	0.0	0.0	4.0	-	0.0
	680/715	0.0	0.0	0.0	0.0	-

Table 3. Spectral cross-talk parameters.

12) Snap-In 2 (green). In "Test Steps" tab, enter the PCR protocol (Table 4).

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 4. PCR protocol.

13) Snap-In 4 (blue). In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 5).

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 5. 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.

14) Snap-In 4 (blue). In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 6), 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 6. Spectral cross-talk parameters.

15) Snap-In 4 (blue). In "Test Steps" tab, enter the PCR protocol (Table 7).

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 7. PCR protocol.

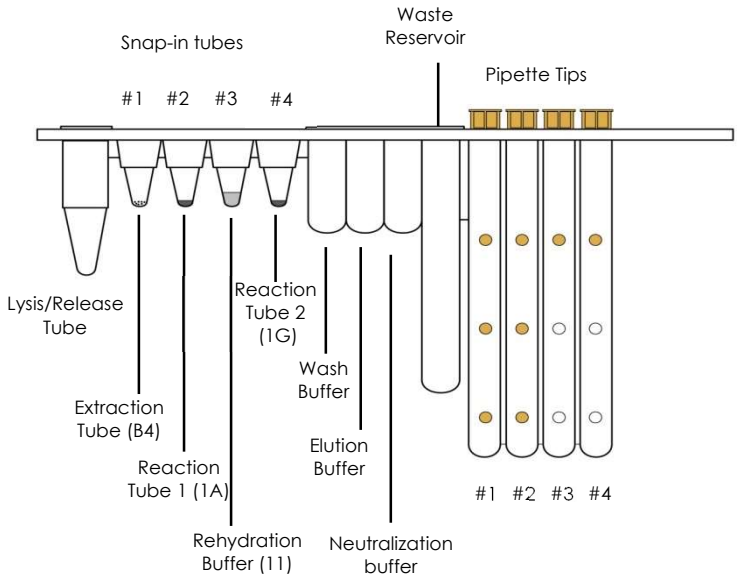
16) Click the "Save Test" button.

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 *Flu A, Flu B & RSV* reaction tubes (red or 1A 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.
4) Remove the required number of Rehydration Buffer tubes (orange or 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 product is at the bottom of the tube.

5) Determine and separate the appropriate number of *SARS-CoV-2 (N1 + N2)* reaction tubes (green or 1G foil) and snap into their corresponding positions in the strip (Snap position 4, blue 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.

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 VSARSCoV2,FluA+B,RSV (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 Worklist, either by scanning the barcode with the scanner or by manual entry.
5) Fill the Specimen/Patient ID and/or Accession window of the Worklist 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 2). 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 Tables 8 and 9.

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 tables:

a. *Flu A, Flu B & RSV* reaction tube: Snap-In 2

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

Table 8. Sample interpretation *Flu A, Flu B & RSV* reaction tube

+: Amplification occured
-: No amplification occured

1 A sample is considered positive if the Ct value obtained is less than 40. The internal control may or may not show an amplification signal, because a high copy number of target can cause preferential amplification of target-specific nucleic acids instead of the internal control. In these cases, the detection of the IC is not necessary.

2 A sample is considered negative, if the sample shows no amplification signal in the detection system but the internal control is positive (Ct less than 40). An inhibition of the PCR reaction can be excluded by the amplification of internal control. In case of unresolved results (UNR), absence of internal control signal in negative sample it is recommended to repeat the assay.

b. *SARS-CoV-2 (N1 + N2)* reaction tube: Snap-In 4

SARS-CoV-2 (N2 target) (475/520)	Endogenous Internal Control (530/565)	SARS-CoV-2 (N1 target) (630/665)	Interpretation
+	+/-[3]	+	**SARS-CoV-2 *N gene* RNA Detected [3]**
+[4]	+/-[3]	-	**SARS-CoV-2 *N gene* RNA Detected [3,4]**
-	+/-[3]	+[4]	**SARS-CoV-2 *N gene* RNA Detected [3,4]**
-	+[5]	-	**SARS-CoV-2 *N* gene RNA Not Detected[5]**
-	-[5]	-	**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.[5]**
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 9. Sample interpretation *SARS-CoV-2 (N1 + N2)* reaction tube

+: Amplification occurred

-: No amplification occurred

3 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.

4 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.

5 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/ oropharyngeal swab 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, Flu A, Flu B and/or RSV 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* (SARS-CoV-2) used in VIASURE *SARS-CoV-2, Flu (A+B) & RSV* 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, Flu and/or RSV 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.
  - Failure to follow instructions for use and the assay procedure.
- In *SARS-CoV-2 (N1 + N2)* reaction tube, a single-target site amplification or even random positive results is suggestive of slightly different amplification yield of the target site 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.
- Some samples (in *SARS-CoV-2 (N1 + N2)* reaction tube) 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, Flu and/or RSV 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.
- Negative results do not preclude SARS-CoV-2, Flu and/or RSV 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 and novel Influenza A strain 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, Flu and/or RSV 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, Flu (A+B) & RSV* 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, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System contains an internal control in each *Flu A, Flu B & RSV* reaction tube and an endogenous internal control in each *SARS-CoV-2 (N1 + N2)* 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, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System was tested individually in each reaction tube.

The clinical performance of *Flu A, Flu B & RSV* reaction tube was tested using 344 respiratory specimens (oropharyngeal swabs) from symptomatic patients. These results were compared with those obtained with a molecular detection method (cobas® Influenza A/B & RSV (Roche)).

The results were as follows:

***Flu A,Flu B & RSV* reaction tube**	**cobas® Influenza A/B & RSV (Roche)**			
		+	**-**	**Total**
	+	157	2*	159
	-	7*	178	185
	Total	164	180	344

Table 10. Comparative results for Flu A.

Positive percent agreement is >96% and negative percent agreement is >99%.

**The low amount of template RNA in this respiratory sample is below the detection limit of the method used.*

***Flu A, Flu B & RSV* reaction tube**	**cobas® Influenza A/B & RSV (Roche)**			
		+	**-**	**Total**
	+	99	4*	103
	-	1*	240	241
	Total	100	244	344

Table 11. Comparative results for Flu B.

Positive percent agreement is >99% and negative percent agreement is >98%.

**The low amount of template RNA in this respiratory sample is below the detection limit of the method used.*

***Flu A, Flu B & RSV* reaction tube**	**cobas® Influenza A/B & RSV (Roche)**			
		+	**-**	**Total**
	+	22	4*	26
	-	3*	315	318
	Total	25	319	344

Table 12. Comparative results for RSV.

Positive percent agreement is >88% and negative percent agreement is >99%.

*The low amount of template RNA in this respiratory sample is below the detection limit of the method used.

The clinical performance of *SARS-CoV-2 (N1 + N2)* reaction tube was tested using 254 respiratory samples (nasopharyngeal swabs in Vircell Transport medium) from patients with clinical suspicion of COVID-19 disease or other similar respiratory diseases. The results were compared with those obtained with the clinical diagnosis performed with Simplexa™ COVID-19 Direct assay with discrepant analysis performed with the Charité protocol.

SARS-CoV-2 (N1 + N2) reaction tube	Alternative RT-PCR assays			
		+	-	Total
	+	63	2*	65
	-	0	189	189
	Total	63	191	254

Table 13. Comparative results for SARS-CoV-2.

*Initial diagnose of one of the two samples was invalid and reported to the patient as positive for prevention and quarantine period.

SARS-CoV-2 (N1 + N2) reaction tube detected two positive samples that were not detected using Simplexa™ COVID-19 Direct assay and the Charité protocol.

The Positive Percent Agreement (PPA) and the Negative Percent Agreement (NPA) for *SARS-CoV-2 (N1 + N2)* reaction tube are >99% and 98%, respectively.

Results show high agreement to detect SARS-CoV-2, Flu A, Flu B and/or RSV viruses using VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytical sensitivity

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System has a detection limit of ≥ 10 genome copies per reaction for Flu A, ≥ 20 genome copies per reaction for Flu B, ≥ 2 genome copies per reaction for RSV and ≥ 5 genome copies per reaction for SARS-CoV-2 with a positive rate of ≥ 95% (Figures 2, 3, 4, 5 and 6).

Figure 2. Dilution series of Flu A ($2*10^6$-$2*10^1$ copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).

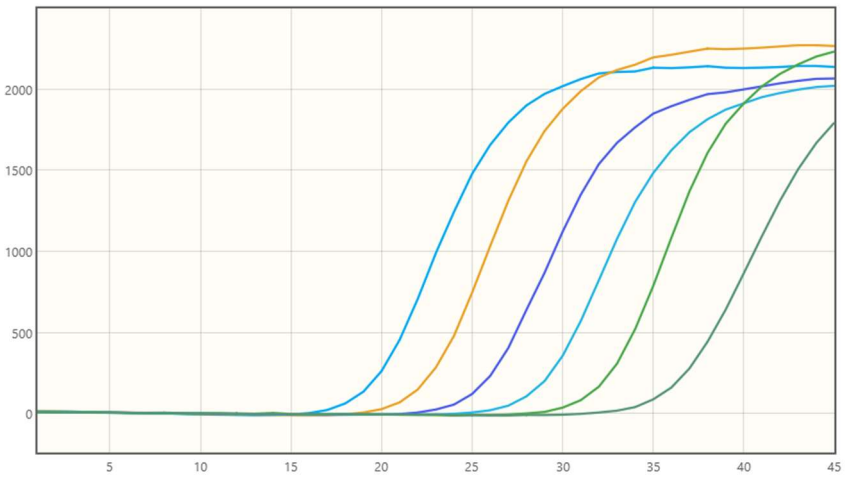


Figure 3. Dilution series of Flu B ($2*10^6$-$2*10^1$ copies per reaction) template run on the BD MAX™ System (585/630 (ROX) channel).

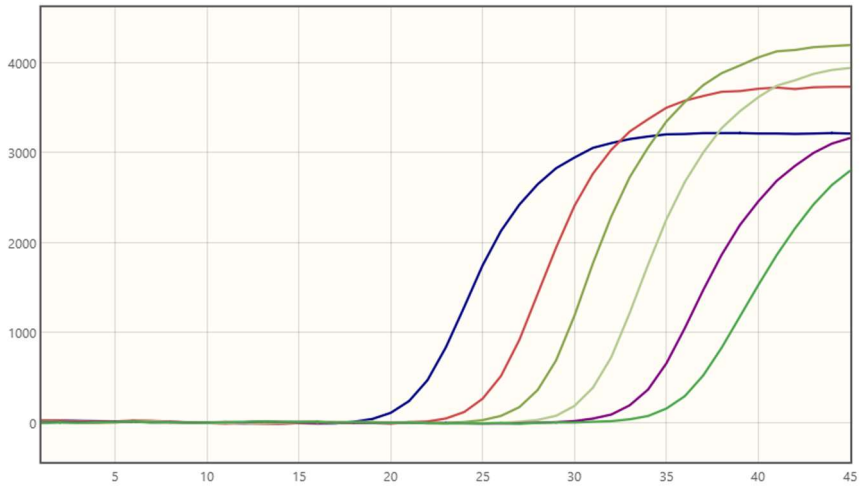


Figure 4. Dilution series of RSV ($2*10^6$-$2*10^1$ copies per reaction) template run on the BD MAX™ System (630/665 (Cy5) channel).

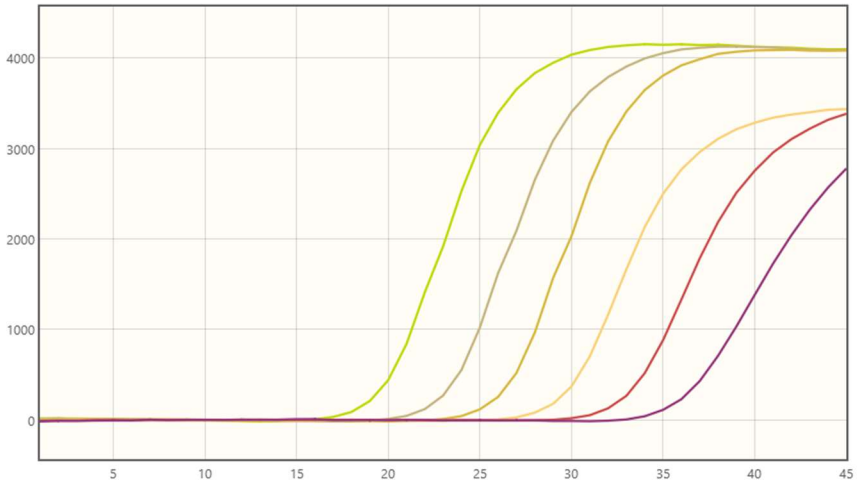


Figure 5. Dilution series of SARS-CoV-2 *(N1 + N2)* ($9.9*10^4$-$9.9*10^0$ and $5.0*10^0$ genome copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).

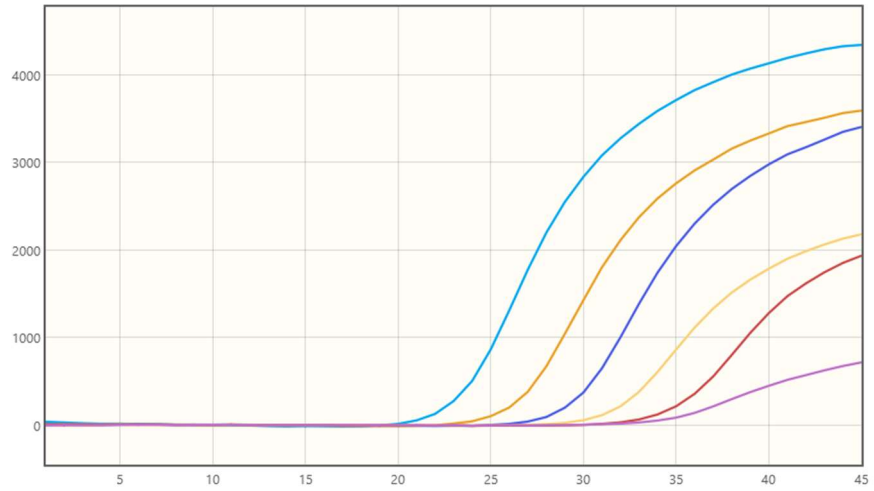
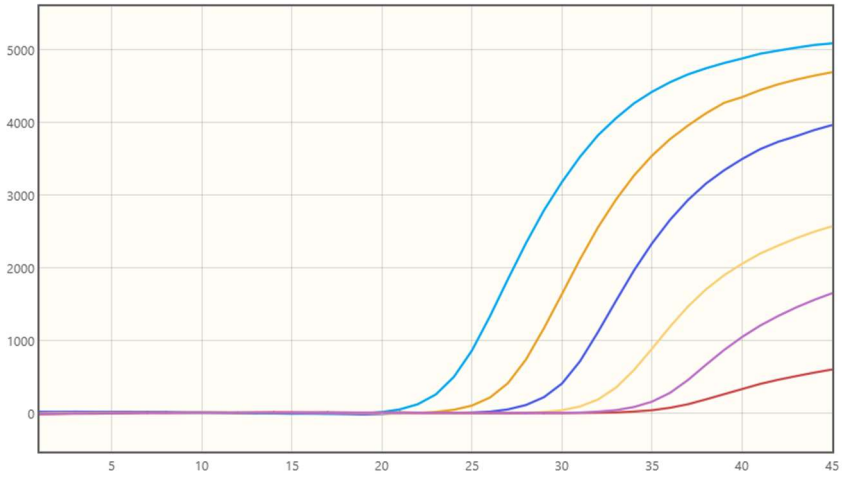


Figure 6. Dilution series of SARS-CoV-2 *(N1 + N2)* ($9.9*10^4$-$9.9*10^0$ and $5.0*10^0$ genome copies per reaction) template run on the BD MAX™ System (630/665 (Cy5) channel).



12.3. Analytical specificity

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

Cross-reactivity testing					
Human Adenovirus types 1-5, 8, 15, 31, 40 and 41	-	Influenza A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a)	-/+	Influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2) virus	-/+
Bocavirus	-	Influenza A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a)	-/+	Influenza A/Chicken/Myanmar/433/2016 (H9N2) virus	-/+
Bordetella bronchiseptica	-	Influenza A/Newcastle/607/2019 (H3N2) virus	-/+	Influenza A/Hong Kong/1073/99 (H9N2) virus	-/+
Bordetella holmesii	-	Influenza A/New York/39/2012 (H3N2) virus	-/+	Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26 virus	-/+
Bordetella parapertussis	-	Influenza A/Ohio/2/2012 (H3N2) virus	-/+	Influenza B/Brisbane/60/2008 virus	-/+
Bordetella pertussis	-	Influenza A/Perth/1001/2018 (H3N2) virus	-/+	Influenza B/Colorado/6/2017 virus	-/+
Chlamydia caviae	-	Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2) virus	-/+	Influenza B/Malaysia/2506/2004 virus	-/+
Chlamydia psittaci genotype A and C	-	Influenza A/South Australia/55/2014 (H3N2) virus	-/+	Influenza B/Maryland/15/2016 virus	-/+
Chlamydophila pneumoniae CM-1	-	Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus	-/+	Influenza B/Netherlands/207/06 virus	-/+
Human coronavirus 229E, OC43, NL63 and HKU1	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-/+	Influenza B/Netherlands/2518/2016 (clade 1A) virus	-/+

Cross-reactivity testing					
MERS Coronavirus	-	Influenza A/Texas/50/2012 (H3N2) virus	-/+	Influenza B/Nevada/3/2011 virus	-/+
SARS Coronavirus Strain Frankfurt 1	-	Influenza A/Thüringen/5/2017 (H3N2) virus (Clade 3C2a.1)	-/+	Influenza B/New Jersey/1/2012 virus	-/+
SARS-CoV-2 strain BetaCoV/Germany/BavPat1/2020 p.1	-/+	Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C) virus	-/+	Influenza B/Texas/02/2013 virus	-/+
SARS-CoV-2 strain 2019-nCoV/Italy-INMI1	-/+	Influenza A/Victoria/210/2009(H3N2) virus	-/+	Influenza B/Townsville/8/2016 virus	-/+
SARS-CoV-2 isolate Australia/VIC01/2020	-/+	Influenza A/Victoria/361/2011 (H3N2) virus	-/+	Influenza B/Canberra/11/2016 virus	-/+
SARS-CoV-2 isolate Wuhan-Hu-1	-/+	Influenza A/Victoria/361/2011 IVR-165 (H3N2) virus	-/+	Influenza B/Florida/4/2006 virus	-/+
SARS-CoV-2 strain 2019nCoV/USAWA1/2020	-/+	Influenza A/Anhui/01/2005 (H5N1) virus	-/+	Influenza B/Florida/07/2004 virus	-/+
Enterovirus 68 and 71	-	Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1) virus	-/+	Influenza B/Guangdong/120/2000 virus	-/+
Enterovirus Echovirus 11 and 30	-	Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1) virus	-/+	Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus	-/+
Enterovirus Coxsackievirus A24, A9 and B3	-	Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1) virus	-/+	Influenza B/ Jiangsu/10/2003 virus	-/+
Haemophilus influenzae MinnA	-	Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus	-/+	Influenza B/Massachusetts/2/2012 virus	-/+
Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09 virus	-/+	Influenza A/chicken/Yunnan/1251/2003 (H5N1) virus	-/+	Influenza B/Netherlands/365/2016 (clade 3) virus	-/+
Influenza A/California/7/2009(H1N1)pdm09 virus	-/+	Influenza A/common magpie/Hong Kong/645/2006 (H5N1) virus	-/+	Influenza B/Phuket/3073/2013 virus	-/+
Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09 virus	-/+	Influenza A/duck/Hunan/795/2002 (H5N1) virus	-/+	Influenza B/Texas/06/2011 virus	-/+
Influenza A/Massachusetts/15/2013 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/321/2007 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 virus	-/+
Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 BX-41A virus	-/+
Influenza A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1)	-/+	Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1) virus	-/+	*Legionella bozemanii*	-
Influenza A/New Caledonia/20/99(H1N1) virus	-/+	Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 virus	-/+	*Legionella dumoffii*	-
Influenza A/New York/18/2009 (H1N1)pdm09 virus	-/+	Influenza A/Hong Kong/213/2003 (H5N1) virus	-/+	*Legionella longbeachae*	-

Cross-reactivity testing					
Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-/+	Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus	-/+	*Legionella micdadei*	-
Influenza A/Sydney/134/2018 (H1N1)pdm09 virus	-/+	Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1) virus	-/+	*Legionella pneumophila*	-
Influenza A/Victoria/2040/2018 (H1N1)pdm09 virus	-/+	Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1) virus	-/+	Human metapneumovirus A and B	-
Influenza A/PR/8/34 (H1N1) virus	-/+	Influenza A/Vietnam/1194/2004 (H5N1) virus	-/+	*Moraxella catarrhalis*	-
Influenza A/Brisbane/117/2018 (H3N2) virus	-/+	Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1) virus	-/+	*Mycoplasma pneumoniae*	-
Influenza A/Brisbane/1028/2017 (H3N2) virus	-/+	Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1) virus	-/+	*Mycobacterium tuberculosis* not rifampin resistant	-
Influenza A/Fujian/411/2002 (H3N2) virus	-/+	Influenza A/Whooper Swan/R65/2006 (H5N1) virus	-/+	Human parainfluenza 1, 2, 3 and 4 viruses	-
Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2) virus	-/+	Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus	-/+	*Pneumocystis jirovecii* Type A1 and g885652	-
Influenza A/Hong Kong/4801/2014 (H3N2) virus	-/+	Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3) virus	-/+	Human rhinovirus type C	-
Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-/+	Influenza A/Duck/Lao/XBY004/2014 (H5N6) (Clade 2.3.4.4) virus	-/+	*Staphylococcus aureus* subsp. *aureus*	-
Influenza A/Indiana/8/2011 (H3N2)v virus	-/+	Influenza A/DE-SH/Reiherente/AR8444/2013 (H5N8) virus	-/+	*Staphylococcus epidermidis*	-
Influenza A/Indiana/10/2011 (H3N2)v virus	-/+	Influenza A/Turkey/Germany/R2485-86/2014 (H5N8) virus	-/+	*Streptococcus pneumoniae* Z022	-
Influenza A/Kansas/14/2017 (H3N2) virus	-/+	Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2) virus	-/+	*Streptococcus pyogenes*	-
Influenza A/Kansas/14/2017, NYMC X-327 (H3N2) virus	-/+	Influenza A/Mallard/Netherlands/2/2009 (H7N7) virus	-/+	*Streptococcus salivarius*	-
Influenza A/Kumamoto/102/2002 (H3N2) virus	-/+	Influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1 virus	-/+	Respiratory syncytial virus (RSV) A and B (strain CH93(18)-18)	-/+
Influenza A/Minnesota/11/2010 (H3N2)v virus	-/+	Influenza A/Anhui/1/2013 (H7N9) virus	-/+	Human Respiratory Syncytial Virus strain Long	-/+
Influenza A/Minnesota/11/2010 X203 (H3N2)v virus	-/+	Influenza A/Guangdong/17SF003/2016 (H7N9) virus	-/+		

Table 14. Reference pathogenic microorganisms used in this study.

12.4. Analytical reactivity

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **SARS-CoV-2** 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, synthetic RNA controls for two variants of the SARS-CoV-2 virus: MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020) and MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1), showing positive result.

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **Influenza A** was evaluated against RNA extracted from the following strains: Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09 virus, Influenza A/California/7/2009(H1N1)pdm09 virus, Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09 virus, Influenza A/Massachusetts/15/2013 (H1N1)pdm09 virus, Influenza A/Michigan/45/2015 (H1N1)pdm09 virus, Influenza A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1), Influenza A/New Caledonia/20/99(H1N1) virus, Influenza A/New York/18/2009 (H1N1)pdm09 virus, Influenza A/Singapore/GP1908/2015 virus, IVR-180 (H1N1)pdm09 virus, Influenza A/Sydney/134/2018 (H1N1)pdm09 virus, Influenza A/Victoria/2040/2018 (H1N1)pdm09 virus, Influenza A/PR/8/34 (H1N1) virus, Influenza A/Brisbane/117/2018 (H3N2) virus, Influenza A/Brisbane/1028/2017 (H3N2) virus, Influenza A/Fujian/411/2002 (H3N2) virus, Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2) virus, Influenza A/Hong Kong/4801/2014 (H3N2) virus, Influenza A/Hong Kong/4801/2014 NYMC X-263B (H3N2) virus, Influenza A/Indiana/8/2011 (H3N2)v virus, Influenza A/Indiana/10/2011 (H3N2)v virus, Influenza A/Kansas/14/2017 (H3N2) virus, Influenza A/Kansas/14/2017, NYMC X-327 (H3N2) virus, Influenza A/Kumamoto/102/2002 (H3N2) virus, Influenza A/Minnesota/11/2010 (H3N2)v virus, Influenza A/Minnesota/11/2010 X203 (H3N2)v virus, Influenza A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a), Influenza A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a), Influenza A/Newcastle/607/2019 (H3N2) virus, Influenza A/New York/39/2012 (H3N2) virus, Influenza A/Ohio/2/2012 (H3N2) virus, Influenza A/Perth/1001/2018 (H3N2) virus, Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2) virus, Influenza A/South Australia/55/2014 (H3N2) virus, Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus, Influenza A/Switzerland/9715293/2013 (H3N2) virus, Influenza A/Texas/50/2012 (H3N2) virus, Influenza A/Thüringen/5/2017 (H3N2) virus (Clade 3C2a.1), Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C) virus, Influenza A/Victoria/210/2009(H3N2) virus, Influenza A/Victoria/361/2011 (H3N2) virus, Influenza A/Victoria/361/2011 IVR-165 (H3N2) virus, Influenza A/Anhui/01/2005 (H5N1) virus, Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1) virus, Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1) virus, Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1) virus, Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus, Influenza A/chicken/Yunnan/1251/2003 (H5N1) virus, Influenza A/common magpie/Hong Kong/645/2006 (H5N1) virus, Influenza A/duck/Hunan/795/2002 (H5N1) virus, Influenza A/Egypt/321/2007 (H5N1) virus, Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1) virus, Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1) virus, Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 virus, Influenza A/Hong Kong/213/2003 (H5N1) virus, Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus, Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1) virus, Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1) virus, Influenza A/Vietnam/1194/2004 (H5N1) virus, Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1) virus, Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1) virus, Influenza A/Whooper Swan/R65/2006 (H5N1) virus, Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus, Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3) virus, Influenza A/Duck/Lao/XBY004/2014 (H5N6) virus (Clade 2.3.4.4), Influenza A/DE-SH/Reiherente/AR8444/2016 (H5N8) virus, Influenza A/Turkey/Germany/R2485-86/2014 (H5N8) virus, Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2) virus, Influenza A/Mallard/Netherlands/2/2009 (H7N7) virus, Influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1 virus, Influenza A/Anhui/1/2013 (H7N9) virus, Influenza A/Guangdong/17SF003/2016 (H7N9) virus, Influenza A/Chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2) virus,

Influenza A/Chicken/Myanmar/433/2016 (H9N2) virus, Influenza A/Hong Kong/1073/99 (H9N2) virus, Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26 virus, showing positive result.

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **Influenza B** was evaluated against RNA extracted from the following strains: Influenza B/Brisbane/60/2008 virus, Influenza B/Colorado/6/2017 virus, Influenza B/Malaysia/2506/2004 virus, Influenza B/Maryland/15/2016 virus, Influenza B/Netherlands/207/06 virus, Influenza B/Netherlands/2518/2016 (clade 1A) virus, Influenza B/Nevada/3/2011 virus, Influenza B/New Jersey/1/2012 virus, Influenza B/Texas/02/2013 virus , Influenza B/Townsville/8/2016 virus (**B/Victoria lineage**); Influenza B/Canberra/11/2016 virus, Influenza B/Florida/4/2006 virus, Influenza B/Florida/07/2004 virus, Influenza B/Guangdong/120/2000 virus, Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus, Influenza B/Jiangsu/10/2003 virus, Influenza B/Massachusetts/2/2012 virus, Influenza B/Netherlands/365/2016 (clade 3) virus, Influenza B/Phuket/3073/2013 virus, Influenza B/Texas/06/2011 virus, Influenza B/Wisconsin/1/2010 virus, Influenza B/Wisconsin/1/2010 BX-41A virus (**B/Yamagata lineage**), showing positive result.

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **RSV** was confirmed against RNA extracted from RSV A and B (strain CH93(18)-18) and Human Respiratory Syncytial Virus strain Long, showing positive result.

HRVATSKI

1. Namjena

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System je automatski test RT-PCR osmišljen za kvalitativnu detekciju i diferencijaciju RNK iz virusa SARS-CoV-2, influence tipa A (Flu A), influence tipa B (Flu B), i/ili humanog respiratornog sincicijskog virusa A/B (RSV) na uzorcima iz dišnog sustava od pojedinaca sa sumnjom na COVID-19 ili drugom infekcijom dišnog sustava od strane njihovog liječnika. Ovaj test je namijenjen za uporabu kao pomagalo u identifikaciju prisutnosti SARS-CoV-2, Flu A, Flu B i/ili RSV virusne RNK. Test koristi BD MAX™ 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™ sustav. RNK se ekstrahira iz uzoraka iz dišnog sustava, amplificirana uporabom tehnike RT-PCR te detektirana uporabom fluorescentne sonde za boju reporteta specifične za SARS-CoV-2, Flu A, Flu B a/ili RSV.

2. Sažetak i objašnjenje

Koronavirusi su grupa ovijenih, nesegmentiranih, pozitivno usmjerenih RNK virusa koji spadaju u obitelj *Coronaviridae*. Poznato je šest vrsta koronavirusa koji uzrokuju bolesti u ljudi. 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. 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.

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. 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.

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. 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. 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. Manje česti simptomi su grlobolja, začepljen nos, glavobolja, proljev, mučnina i povraćanje. Prijavljeni su također i gubitak mirisa (anozmija) ili gubitak okusa (ageuzija) prethode nastupu respiratornih simptoma. 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.

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) 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 i drugih respiratornih virusa, poput gripe i RSV.

Virusi gripe pripadaju rodu *Orthomyxoviridae* i izazivaju većinu virusnih infekcija donjih dišnih puteva. Gripa tipa A i gripa tipa B predstavljaju značajan uzrok poboljevanja i smrtnosti diljem svijeta, uzevši u obzir da su starije osobe i osobe s narušenim imunološkim sustavom naročito izložene riziku od pojave teške bolesti i komplikacija kao što je pneumonija. Kod pogođenih osoba mogu se javiti neki ili svi sljedeći simptomi: vrućica ili grozničav osjećaj/zimica, kašalj, grlobolja, začepljen nos i iscjedak iz nosa, mijalgija, glavobolje i anoreksija. Virusi gripe mogu se prenositi s jedne osobe na drugu na dva različita načina: putem zraka (krupne kapljice i aerosoli koji se prenose kihanjem i kašljem) te izravnim ili neizravnim kontaktom.

Virusi gripe tipa A i B predstavljaju nerazvijene viruse s jednolančanom RNK koji sadrže osam segmentiranih lanaca genomske RNK koja tipično šifrira 11 ili 12 virusnih proteina. Dodatni virusni omotač, dobiven iz membrane stanice domaćina, sastoji se od lipidnog dvosloja koji sadrži transmembranske proteine, poput hemaglutinina (HA) i neuraminidaze (NA) te matrične proteine M1 i M2. Virusi gripe tipa A dodatno se klasificiraju u podtipove na osnovu antigenosti njihovih „HA" i „NA" molekula, dok se gripa tipa B dijeli u 2 antigenski i genetski različite linije: Victoria i Yamagata.

Humani respiratorni sincicijski virusi A i B (RSV) pripadaju rodu *Paramyxoviridae* i predstavljaju najvažnije virusne uzročnike akutnih respiratornih infekcija. RSV je nesegmentirani virus s jednolančanom linearnom RNK negativnog polariteta s dodatnim omotačem. Respiratorni sincicijski virus čest je nositelj respiratornih infekcija koji izaziva bronhitis, pneumoniju i kronične opstruktivne plućne infekcije kod osoba svih starosnih skupina. Kod pogođenih osoba često se javljaju neki ili svi sljedeći simptomi: curenje nosa, vrućica niskog stupnja, kašalj, grlobolja, glavobolja i piskutanje prilikom disanja. RSV se prenosi putem krupnih kapljica nazofaringealnog sekreta zaraženih osoba, bliskog kontakta ili zarazom nakon dolaska u dodir s kontaminiranim površinama.

Uspostavljanje dijagnoze može se pokazati problematičnim, jer širok spektar patogena može izazvati akutne respiratorne infekcije koje imaju slične kliničke sindrome. Testovi lančanom reakcijom polimeraze u stvarnom vremenu pokazali su se kao osjetljiv i specifičan dijagnostički alat za detekciju virusa SARS-CoV-2, Flu A, Flu B i RSV.

3. Načelo postupka

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System osmišljen je za identifikacij virusa SARS-CoV-2, Flu A, Flu B i /ili RSV u respiratornim uzorcima. 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 RNK, vrši se njena transkripcija čime se dobiva komplementarna DNK zahvaljujući reverznoj transkriptazi, nakon koje slijedi amplifikacija konzervirane regije gena *N* (N1 i N2) za SARS-CoV-2, konzervirane regije gena M1 za gripu tipa A i gripu tipa B te konzervirane regije gena N za virus RSV primjenom specifičnih početnica i fluorescentno obojanih sondi.

Komplet VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System temelji se na aktivnosti 5´ eksonukleaze 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, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System sastoji se od dvije različite reakcijske epruvete. Jedna od epruveta detektira i razlikuje RNK od gripe A, gripe B i/ili virusa RSV (prozirno crvena ili 1A folija), a druga epruveta detektira specifično RNK od SARS-CoV-2 (prozirno zelena ili 1G folija). Svaka epruveta sadrži sve komponente potrebno za PCR test u stvarnom vremenu (specifične primere/sonde, dNTPS-ove, pufere, polimeraze, reverzne transkriptaze) u stabiliziranom formatu kao i unutarnju kontrolu (endogenu u reakcijskoj epruveti SARS-CoV-2) za nadzor procesa ekstrakcije i/ili inhibicije aktivnosti polimeraze. SARS-CoV-2 test koristi humani domaćinski gen kao endogenu unutarnju kontrolu (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. Svaka ciljna RNK amplificira se i detektira u specifičnim kanalima (475/520, 585/630, i/ili 630/665) i unutarnjom kontrolom (IC) u kanalu 530/565. Kod gripe tipa A, tipa B i/ili RSV testa, RNK virusa gripe tipa A je pojačana i otkrivena u kanalu 475/520, RNK virusa gripe tipa B u kanalu 585/630, RNK virusa RSV u kanalu 630/665, a unutarnja kontrola (IC) u kanalu 530/565. U testu SARS CoV-2, N2 cilj je amplificiran i detektiran u kanalu 475/520, N1 cilj u kanalu 630/665 i endogena unutarnja kontrola (IC) u kanalu 530/565.

4. Reagensi koji se isporučuju

Komplet VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System uključuje sljedeće materijale i reagense opisane u Tablici 1:

Referenca	Reagens/materijal	Opis	Boja/crtični kod	Količina
VS-ABR212R	*Flu A, Flu B & RSV* reaction tube	Smjesa enzima, sondi/početnica, pufera, dNTP-ova, stabilizatora i unutarnje kontrole u stabiliziranom obliku	Prozirno Crvena ili 1A folija	2 vrećice s 12 epruveta
VS-NCO312	*SARS-CoV-2 (N1 + N2)* reaction tube	Smjesa enzima, sondi za početnice, pufera, dNTP-ova, stabilizatora i endogene unutarnje kontrole u stabiliziranom obliku	Prozirno Zelena ili 1G folija	2 vrećice s 12 epruveta
VS-RB09	Rehydration Buffer tube	Otopina za rekonstituciju stabiliziranog proizvoda	Prozirno Narančasta ili 11 folija	1 vrećica s 24 epruvete

Tablica 1. Reagensi i materijali osigurani u kompletu za detekciju VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System with cat. N°. VS-FNR124 (444217).

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, Flu (A+B) & RSV* 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, Flu (A+B) & RSV* 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™ sustava. 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.
- Pazite da koristtie epruvetu za određivanje RNK influence tipa A, influence tipa B te RSC u Snap-lin 2 (zeleni položaj) te drugu epruvetu za određivanje RNK SARS-CoV-2 u Snap-In 4 (plavi položaj). Pazite da ih ne pomiješate tijekom cijelog procesa.
- 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 u 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 niti pušiti u radnom prostoru. Operite ruke nakon što završite test.
- Uzorci se moraju smatrati potencijalno zaraznim, 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, pohrane, tretiranja i odlaganja uzoraka u otpad.
- Preporučuje se redovita dekontaminacija opreme koja se često koristi, naročito mikropipeta i radnih površina.
- Posavjetujte se s Korisničkim priručnikom za BD MAX™ sustav u vezi s dodatnim upozorenjima, mjerama opreza i postupcima.

8. Postupak

8.1. PRIKUPLJANJE, POHRANA I TRANSPORT UZORAKA

Komplet za detekciju VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System validiran je na nazofaringealnom/orofaringelanom brisu prikupljenom u virusnom transportnom mediju (VTM) Vircell S.L., Španjolska).

Drukčije vrste uzoraka iz nazofaringealnih/orofaringealnih brisova u VTM-u mora validirati korisnik.

Prikupljanje, pohrana i transport uzoraka treba obavljati u uvjetima koje je validirao korisnik. U globalu, respiratorne 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 48 sati u skladu s lokalnim i nacionalnim propisima za transport patogenih materijala. Za dugotrajni transport (duži od 48 sata) preporučujemo otpremanje na temperaturi od ≤-20 °C. Preporučuje se upotreba svježih uzoraka za test. Uzorci se mogu čuvati na temperaturi od 2 °C do 8 °C tijekom 48 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.

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.

1. Pipetom prenesite 400 µl nazofaringealnog/orofaringealnog brisa prikupljenog u virusnom transportnom mediju (VTM) u BD MAX™ TNA-3 Sample Buffer Tube te je zatvorite čepom s membranom. Izmiješajte uzorak vrtloženjem pri visokoj brzini tijekom 1 minute. Pređite na rad sa BD MAX™ sustavom.

Napomena: *Flu A, Flu B & RSV* reaction tube validirana je volumenom uzorka od 200 do 400 µL a reakcijska epruveta *SARS-CoV-2 (N1 + N2)* reaction tube volumenom uzorka od 400 do 750 µL.

8.3. PROTOKOL ZA PCR

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

8.3.1. Izrada PCR test programa za VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System.

Napomena: Ako ste već kreirali test za komplet VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection kit, možete preskočiti korak 8.3.1 i preći izravno na 8.3.2.

1) Na zaslonu „Run" (Pokreni) na BD MAX™ sustavu odaberite karticu „Test Editor" (Uređivač testa).
2) Kliknite na gumb „Create" (Kreiraj).
3) Na kartici Basic Information (Osnovne informacije) unutar prozora "Test Name" (Naziv testa), imenujte svoj test: tj. VIASURE *SARS-CoV-2, Flu (A+B) & RSV* (VSARSCoV2,FluA+B,RSV).
4) S padajućeg izbornika „Extraction Type" (Vrsta ekstrakcije) odaberite „ExK TNA-3".
5) Na padajućem izborniku "Master Mix Format" (Glavni format smjese) odaberite "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Dvojna glavna smjesa koncentriranog liofilizata MM s rehidracijskim puferom (tip 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 Barcode: 1A (u vezi reakcijske erpuvete *Flu A, Flu B & RSV* reaction tube)
    b. Snap-In 3 Barcode: 11 (u vezi epruvete s Rehydration Buffer tube (rehidracijskim puferom))
    c. Snap-In 4 Barcode: 1G (u vezi reakcijske epruvete *SARS-CoV-2 (N1 + N2)* reaction tube)
9) "PCR Settings" (PCR postavke) i "Test Steps" (Testni koraci) moraju biti ispunjeni za Snap-In 2 (zelene) i Snap-In 4 (plave) položaje.
10) Snap-In 2 (zeleno). Na kartici „PCR settings" (Postavke za PCR) unesite sljedeće parametre: „Channel Settings" (Postavke kanala), „Gains" (Dobit) i „Threshold" (Granica) (Tablica 2).

Channel (Kanal)	Alias (Drugi nazivi)	Gain (Dobit)	Threshold (Prag)	Ct Min (Ct Min)	Ct Max (Ct Maks)
475/520 (FAM)	Flu A	60	100	0	40
530/565 (HEX)	IC	80	300	0	40
585/630 (ROX)	Flu B	60	200	0	40
630/665 (Cy5)	RSV	60	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Tablica 2. 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.

11) Snap-In 2 (zeleno). Na kartici „PCR settings“ (Postavke za PCR) unesite i sljedeće parametre: „Spectral Cross Talk“ (Tablica 3)

		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	-	0,0	0,0	0,0	0,0
	530/565	0,0	-	2,0	0,0	0,0
	585/630	0,0	0,0	-	0,0	0,0
	630/665	0,0	0,0	4,0	-	0,0
	680/715	0,0	0,0	0,0	0,0	-

Tablica 3. Parametri spektralnog preklapanja signala

12) Snap-In 2 (zeleno). Na kartici „Test Steps“ (Koraci testa) unesite protokol za PCR (Tablica 4).

Step Name (Naziv koraka)	**Profile Type (Vrsta profila)**	**Cycles (Ciklusi)**	**Time (s) (Vremena(s))**	**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 4. Protokol za PCR.

13) Snap-In 4 (plavo). Na kartici „PCR settings“ (Postavke za PCR) unesite sljedeće parametre: „Channel Settings“ (Postavke kanala), „Gains“ (Dobit) i „Threshold“ (Granica) (Tablica 5).

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 5. 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.

14) Snap-In 4 (plavo). Na kartici „PCR settings“ (Postavke za PCR) unesite i sljedeće parametre: „Spectral Cross Talk“ (Tablica 6)

		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 6. Parametri spektralnog preklapanja signala

15) Snap-In 4 (plavo). Na kartici „Test Steps" (Koraci testa) unesite protokol za PCR (Tablica 7).

Step Name (Naziv koraka)	**Profile Type (Vrsta profila)**	**Cycles (Ciklusi)**	**Time (s) (Vremena(s))**	**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 7. Protokol za PCR.

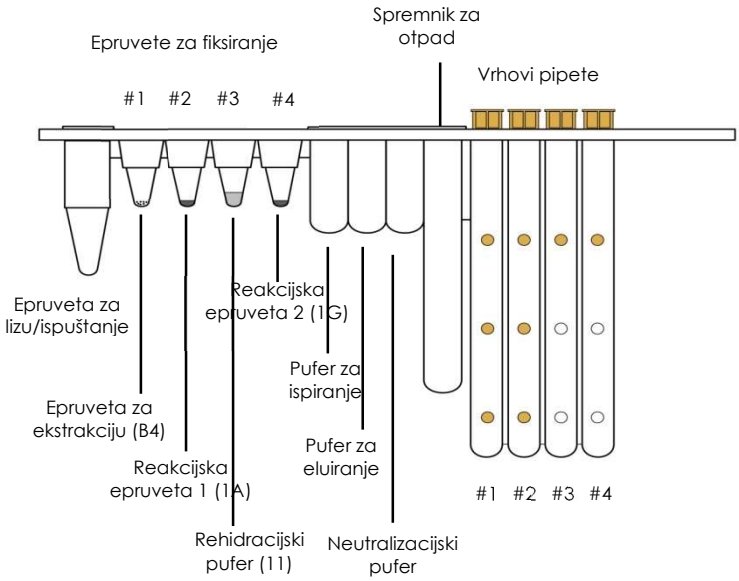
16) Kliknite na gumb „Save Test" (Spremi test).

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 od 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 extraction tube (B4) iz njihove zaštitne vrećice. Postavite epruvetu/e za ekstrakciju (bijela folija) u njihove odgovarajuće položaje u TNA traku (položaj 1 s oznakom u boji na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite vrećicu patentnom zatvaračem.
3) Odredite i razdvojite odgovarajući broj *Flu A, Flu B & RSV* reaction tube (crvena folija i 1A folija) i postavite ih u njihove odgovarajuće položaje na traci (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.

4) Izvadite odgovarajući broj epruveta Rehydration Buffer tubes (narančasta folija i 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.
5) Odredite i razdvojite odgovarajući broj reakcijskih epruveta *SARS-CoV-2 (N1 + N2)* reaction tube (zelena folija i 1G folija) i postavite ih u njihove odgovarajuće položaje na traci (položaj 4, označen plavom 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.

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“ odaberite VSARSCoV2,FluA+B,RSV (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 epruvete za uzorak s puferom u prozor „Sample tube“ (Epruveta s uzorkom) 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™ sustav (stalak A se nalazi lijevo u odnosu na BD MAX™ sustav, dok se stalak B nalazi na desnoj strani).
8) Stavite potrebni broj patrona BD MAX™ PCR Cartridge(s) u BD MAX™ System.
9) Zatvorite vrata BD MAX™ sustava.
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 2). 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 Tablicama 8 i 9.

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:

a. *Flu A, Flu B & RSV* reaction tube: Snap-In 2

Gripa tipa A (475/520)	Gripa tipa B (585/630)	RSV (630/665)	Unutarnja kontrola (530/565)	Tumačenje
+	+	+	+/-[1]	**Detektiran virus gripe A, gripe B te RSV RNK[1]**
+	-	-	+/-[1]	**Detektirana RNK gripe A, gripe B, a RSV RNK nije detektirana[1]**
+	+	-	+/-[1]	**Detektirana RNK gripe A i gripe B te RSV RNK koja nije detektirana[1]**
+	-	+	+/-[1]	**Detektirana RNK gripe A te RSV RNK, a gripe B RNK koja nije detektirana[1]**
-	+	-	+/-[1]	**Detektirana RNK gripe B, gripe A, a RSV RNK nije detektirana[1]**
-	+	+	+/-[1]	**Detektirana RNK gripe B te virusa RSV RNK, a gripe A nije detektirana[1]**
-	-	+	+/-[1]	**Detektirana RNK RSV, gripe A, a RNK gripe B nije detektirana[1]**
-	-	-	+[2]	**Gripe A, gripe B i RSV RNK nisu detektirane[2]**
-	-	-	-[2]	**Neriješen (UNR) rezultat dobiven u prisutnosti inhibitora reakcije PCR-a ili kada dođe do nekog općeg problema (koji nije pokriven šifrom o pogrešci) pri obradi uzorka i/ili amplifikaciji.[2]**
IND	IND	IND	IND	**Rezultat testa nije moguće utvrditi (IND). Zbog kvara BD MAX™ sustava. Rezultat testa prikazan u slučaju kvara instrumenta povezanog sa šifrom o pogrešci.**
INC	INC	INC	INC	**Nepotpun rezultat testa (INC). Zbog kvara BD MAX™ sustava. Rezultat testa prikazan u slučaju neuspješnog obavljanja postupka.**

Tablica 8. Tumačenje uzorka iz *Flu A, Flu B & RSV* reaction tube

+: Došlo je do amplifikacije
-: Nije došlo do amplifikacije

1 Uzorak se smatra pozitivnim ako je dobivena Ct vrijednost manja od 40. Unutarnja kontrola može ili ne mora pokazati amplifikacijski signal, jer veliki broj kopija ciljne nukleinske kiseline može izazvati preferencijalnu amplifikaciju specifičnih nukleinskih kiselina umjesto unutarnje kontrole. U tim slučajevima nije potrebna detekcija unutarnje kontrole.

2 Uzorak se smatra negativnim ako ne pokazuje amplifikacijski signal u sustavu detekcije, ali je unutarnja kontrola pozitivna (Ct manja od 40). Inhibicija PCR reakcije može se isključiti amplifikacijom interne kontrole u slučaju neriješenih rezultata (UNR) te odsutnosti signala interne kontrole u negativnom uzorku te se preporučuje ponoviti test.

b. *SARS-CoV-2 (N1 + N2)* reaction tube: Snap-In 4

SARS-CoV-2 (N2 cilj) (475/520)	Endogena unutarnja kontrola (530/565)	SARS-CoV-2 (N1 cilj) (630/665)	Tumačenje
+	+/-[3]	+	**SARS-CoV-2 *N genska* RNK detektirana [3]**
+[4]	+/-[3]	-	**SARS-CoV-2 *N genska* RNK detektirana [3,4]**
-	+/-[3]	+[4]	**SARS-CoV-2 *N genska* RNK detektirana [3,4]**
-	+[5]	-	**SARS-CoV-2 *N* genska RNK nije otkrivena[5]**
-	-[5]	-	**Neriješeni (UNR) Rrezultat dobiven u prisutnosti inhibitora reakcije PCR-a ili kada dođe do nekog općeg problema (koji nije pokriven šifrom o pogrešci) pri obradi uzorka i/ili amplifikaciji.[5]**
IND	IND	IND	**Rezultat testa nije moguće utvrditi (IND). Zbog kvara BD MAX™ sustava. Rezultat testa prikazan u slučaju kvara instrumenta povezanog sa šifrom o pogrešci.**
INC	INC	INC	**Nepotpun rezultat testa (INC). Zbog kvara BD MAX™ sustava. Rezultat testa prikazan u slučaju neuspješnog obavljanja postupka.**

Tablica 9. Tumačenje uzorka iz *SARS-CoV-2 (N1 + N2)* reaction tube

+: Došlo je do amplifikacije

-: Nije došlo do amplifikacije

3 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 amolifikaciju ciljno-specifičnih nukleinskih kiselina.

4 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.

5 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 "Neriješen" 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 nazofaringealnim/orofaringealnim brisovima prikupljenima u VTM-u.
- 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 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 SARS-CoV-2, gripom tipa A, gripom tipa B i/ili virusom RSV, bilo u slučaju da uzorci sadrže visoke koncentracije ciljne RNK ili u slučaju kontaminacije izazvane proizvodima koji sadrže PCR iz ranijih reakcija.
- Kombinacije specifične početnice i sonde za detekciju uščuvanih regija *N* gena (SARS-CoV-2) koji se koristi u kompletu VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System osmišljeni su na temelju testa 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 čimbenika te njihovih kombinacija uključujući:
    - nepravilno prikupljanje uzoraka, transport, pohrana i/ili metode rukovanja.
    - nepravilne postupke obrade (uključujući ekstrakciju RNK).
    - Degradacija virusne RNK tijekom 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, gripe i/ili RSV.
    - Virusno opterećenje u uzorku koje je ispod granice detekcije za test:
    - Prisutnost RT-qPCR inhibitora ili drugih tipova interferirajućih tvari.
    - Neuspjeh u pridržavanju uputa za uporavu te prilikom postupka testiranja.
- U *SARS-CoV-2 (N1 + N2)* reaction tube 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.

- Neki uzorci (u *SARS-CoV-2 (N1 + N2)* reaction tube) možda neće iskazati amplifikacijske krivulje *RNase P* zbog niskog broja ljudskih stanica u izvornom kliničkom uzorku. Negativan signal unutarnje kontrole ne isključuje prisutnost SARS-CoV-2, gripe i/ili RSV RNK u kliničkom uzorku.
- Pozitivan rezultat testa ne ukazuje nužno na prisutnost održiv virusa te ne ukazuje da su ti virusi infektivni ili uzročni agensi za kliničke simptome. Međutim, pozitivni rezultat ukazuje na prisutnost ciljnih virusnih sekvenci.
- Negativni rezultati ne isključuju SARS-CoV-2, gripu i/ili RSV infekciju i ne smiju se koristiti kao jedini temelj za odlučivanje o liječenju ili pružanju druge zdravstvene skrbi bolesniku. Nisu određene optimalne vrste te vremenskog određivanje za vršne razine virusa tijekom infekcija uzrokovanih virusom SARS-CoV-2 te novim sojem influence A. 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 SARS-CoV-2, gripu i/ili RSV infekcija potrebno je razmotriti mogućnost lažnog negativnog rezultata i ponovnog testiranja bolesnika.
- U slučaju neriješenih, neutvrdivih ili nepotpunih rezultata primjenom VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System potrebno je ponovno testiranje. Neriješen 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 za detekciju VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System sadrži unutarnju kontrolu u svakoj *Flu A, Flu B & RSV* reaction tube kao endogenu unutarnju kontrolu u svakoj *SARS-CoV-2 (N1 + N2)* reaction tube koja potvrđuje ispravnu učinkovitost tehnike.

12. Radne karakteristike

12.1. Klinička osjetljivost i specifičnost

Klinički učinak PCR kompleta za detekciju u stvarnom vremenu VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System testiran je pojedinačno u svakoj reakcijskoj epruveti.

Klinički učinak kompleta *Flu A, Flu B & RSV* reaction tube testiran je na 344 respiratorna uzorka (brisovi orofaringeusa) od simptomatskih bolesnika. Ti su rezultati uspoređeni s rezultatima dobivenim pomoću metode molekularne detekcije (cobas® Influenza A/B & RSV (Roche)).

Dobiveni su sljedeći rezultati:

Flu A, Flu B & RSV reaction tube	cobas® Influenza A/B & RSV (Roche)			
		+	-	Ukupno
	+	157	2*	159
	-	7*	178	185
	Ukupno	164	180	344

Tablica 10. Komparativni rezultati za gripu tipa A.

Postotak podudarnosti pozitivnih rezultata je >96%, a postotak podudarnosti negativnih rezultata je >99%.

**Niska količina RNK predloška u ovom respiratornom uzorku je ispod granice detekcije za korištenu metodu.*

Flu A, Flu B & RSV reaction tube	cobas® Influenza A/B & RSV (Roche)			
		+	-	Ukupno
	+	99	4*	103
	-	1*	240	241
	Ukupno	100	244	344

Tablica 11. Komparativni rezultati za gripu tipa B.

Postotak podudarnosti pozitivnih rezultata je >99%, a postotak podudarnosti negativnih rezultata je >98%.

**Niska količina RNK predloška u ovom respiratornom uzorku je ispod granice detekcije za korištenu metodu.*

Flu A, Flu B & RSV reaction tube	cobas® Influenza A/B & RSV (Roche)			
		+	-	Ukupno
	+	22	4*	26
	-	3*	315	318
	Ukupno	25	319	344

Tablica 12. Komparativni rezultati za virus RSV.

Postotak podudarnosti pozitivnih rezultata je >88%, a postotak podudarnosti negativnih rezultata je >99 %.

*Niska količina RNK predloška u ovom respiratornom uzorku je ispod granice detekcije za korištenu metodu.

Klinička učinkovitost kompleta *SARS-CoV-2 (N1 + N2)* reaction tube testirana je uporabom 254 uzorka iz dišnog sustava (nazofaringelani brisevi u transportnom mediju Vircell) od pacijenata s kliničkom sumnjom na bolest COVID-19 ili drugu sličnu respiratornu bolest. Rezultati su uspoređeni s onima dobivenim s kliničkom dijagnozom

provedenom sa Simplexa™ COVID-19 Direct assay izravnim testom s diskrepantnom analizom provedenom prema protokolu Charité.

		Alternativni testovi RT-PCR		
		+	-	Ukupno
***SARS-CoV-2 (N1 + N2)* reaction tube**	**+**	63	2*	65
	-	0	189	189
	Ukupno	63	191	254

Tablica 13. Komparativni rezultati za SARS-CoV-2.

*Početna dijagnoza za jedan ili dva uzorka nije bila valjana te je prijavljena pacijentu kao pozitivna u svrhu prevencije i razdoblja karantene.

SARS-CoV-2 (N1 + N2) reaction tube detektirala je dva pozitivna uzorka koji nisu detektirani uporabom testa Simplexa™ COVID-19 Direct assay te protokola Charité.

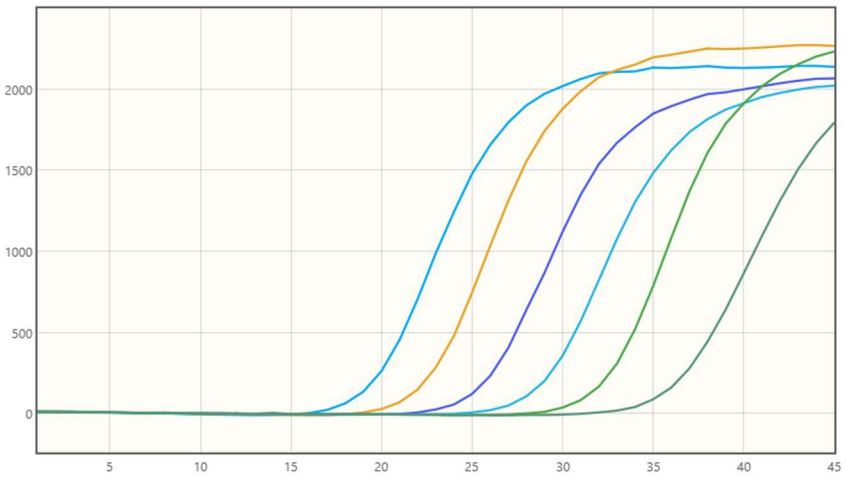
Postotak pozitivnog slaganja (PPA) te postotak negativnog slaganja (NPA) za komplet za *SARS-CoV-2 (N1 + N2)* reaction tube su >99% odnosno 98%.

Rezultati pokazuju visoko slaganje za detekciju SARS-CoV-2, gripe tipa A, gripe tipa B i/ili RSV virusa uporabom kompleta za detekciju VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System.

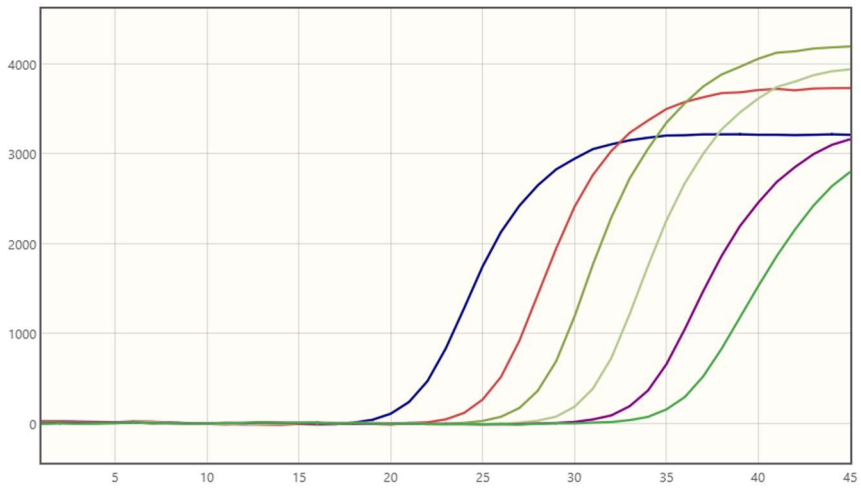
12.2. Analitička osjetljivost

Komplet za detekciju VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System ima limit detekcije od ≥ 10 kopija genoma po reakciji za gripu tipa A, ≥ 20 kopija genoma za gripu tipa B, ≥ 2 kopije genoma po reakciji za RSV te ≥ 5 kopija genoma po reakciji za SARS-CoV-2 s pozitivnom stopom od ≥ 95% (slike 2, 3, 4, 5 i 6).

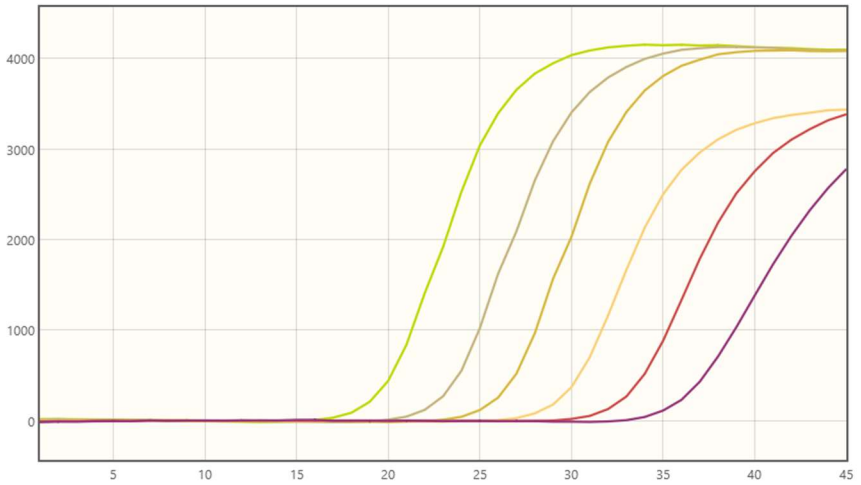
Slika 2. Serija razrjeđivanja predloška gripe tipa A ($2*10^6$-$2*10^1$ kopije/rxn) analizirana na BD MAX™ sustavu (475/520 (FAM) kanal).



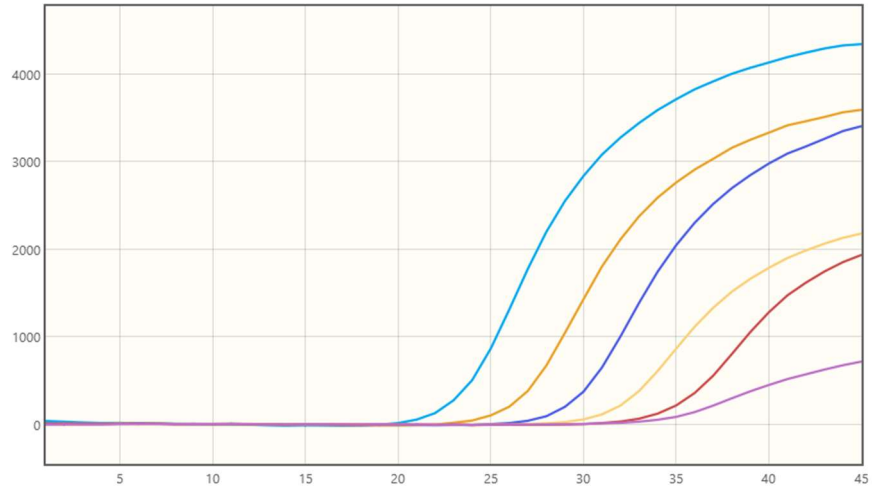
Slika 3. Serija razrjeđivanja predloška gripe tipa B ($2*10^6$-$2*10^1$ kopije po reakciji) analizirana na BD MAX™ sustavu (585/630 (ROX) kanal).



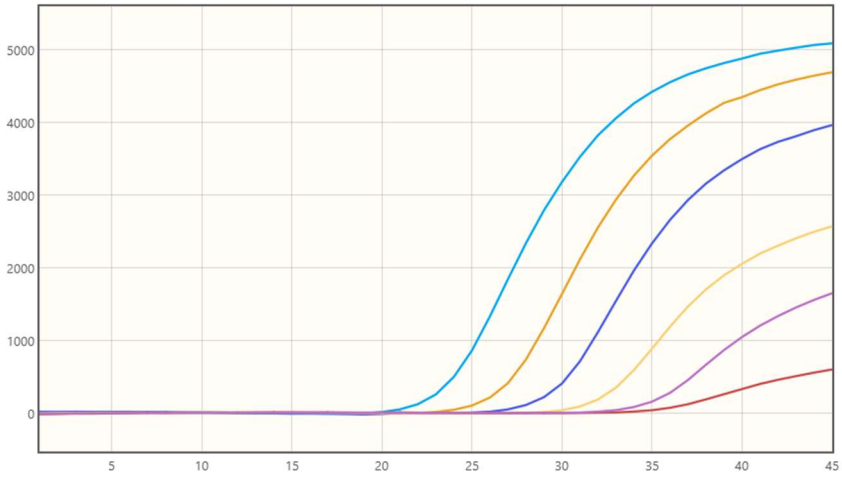
Slika 4. Serija razrjeđivanja predloška RSV ($2*10^6$-$2*10^1$ kopije po reakciji) analizirana na BD MAX™ sustavu (630/665 (Cy5) kanal).



Slika 5. 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) provodi se na sustavu BD MAX™ System (475/520 (FAM) kanal).



Slika 6. 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) provodi se na sustavu BD MAX™ System (630/665 (Cy5) kanal).



12.3. Analitička specifičnost

Specifičnost testa za SARS-CoV-2, Flu (A+B) & RSV potvrđena je testiranjem panela koji se sastoji od različitih mikroorganizama koji predstavljaju najčešće respiratorne patogene. Nije zabilježena križna reaktivnost između bilo kojih od sljedećih testiranih mikroorganizama, izuzev ciljanih patogena svakog testa:

Testiranje unakrsne reaktivnosti					
Ljudski adenovirus tipovi 1-5, 8, 15, 31, 40 i 41	-	Influenza A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a)	-/+	Influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2) virus	-/+
Bocavirus	-	Influenza A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a)	-/+	Influenza A/ chicken /Myanmar/433/2016 (H9N2) virus	-/+
Bordetella bronchiseptica	-	Influenza A/Newcastle/607/2019 (H3N2) virus	-/+	Influenza A/Hong Kong/1073/99 (H9N2) virus	-/+
Bordetella holmesii	-	Influenza A/New York/39/2012 (H3N2) virus	-/+	Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26 virus	-/+
Bordetella parapertussis	-	Influenza A/Ohio/2/2012 (H3N2) virus	-/+	Influenza B/Brisbane/60/2008 virus	-/+
Bordetella pertussis	-	Influenza A/Perth/1001/2018 (H3N2) virus	-/+	Influenza B/Colorado/6/2017 virus	-/+
Chlamydia caviae	-	Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2) virus	-/+	Influenza B/Malaysia/2506/2004 virus	-/+
Chlamydia psittaci genotip A i C	-	Influenza A/ South Australia/55/2014, IVR-175 (H3N2) virus	-/+	Influenza B/Maryland/15/2016 virus	-/+
Chlamydophila pneumoniae CM-1	-	Influenza A/ South Australia/55/2014, IVR-175 (H3N2) virus	-/+	Influenza B/Netherlands/207/06 virus	-/+
Ljudski koronavirus 229E, OC43, NL63 i HKU1	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-/+	Influenza B/Netherlands/2518/2016 (clade 1A) virus	-/+
MERS koronavirus	-	Influenza A/Texas/50/2012 (H3N2) virus	-/+	Influenza B/Nevada/3/2011 virus	-/+

Testiranje unakrsne reaktivnosti					
SARS koronavirus Soj Frankfurt 1	-	Influenza A/Thüringen/5/2017 (H3N2) virus (Clade 3C2a.1)	-/+	Influenza B/New Jersey/1/2012 virus	-/+
SARS-CoV-2 soj BetaCoV/Germany/BavPat1/2020 p.1	-/+	Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C) virus	-/+	Influenza B/Texas/02/2013 virus	-/+
SARS-CoV-2 soj 2019-nCoV/Italy-INMI1	-/+	Influenza A/Victoria/210/2009 (H3N2) virus	-/+	Influenza B/Townsville/8/2016 virus	-/+
SARS-CoV-2 izolat Australia/VIC01/2020	-/+	Influenza A/Victoria/361/2011 (H3N2) virus	-/+	Influenza B/Canberra/11/2016 virus	-/+
SARS-CoV-2 izolat Wuhan-Hu-1	-/+	Influenza A/Victoria/361/2011 IVR-165 (H3N2) virus	-/+	Influenza B/Florida/4/2006 virus	-/+
SARS-CoV-2 soj 2019nCoV/USAWA1/2020	-/+	Influenza A/Anhui/01/2005 (H5N1) virus	-/+	Influenza B/Florida/07/2004 virus	-/+
Enterovirus 68 i 71	-	Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1) virus	-/+	Influenza B/Guangdong/120/2000 virus	-/+
Enterovirus Echovirus 11 i 30	-	Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1) virus	-/+	Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus	-/+
Enterovirus Coxsackievirus A24, A9 i B3	-	Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1) virus	-/+	Influenza B/ Jiangsu/10/2003 virus	-/+
Haemophilus influenzae MinnA	-	Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus	-/+	Influenza B/Massachusetts/2/2012 virus	-/+
Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09 virus	-/+	Influenza A/chicken/Yunnan/1251/2003 (H5N1) virus	-/+	Influenza B/Netherlands/365/2016 (clade 3) virus	-/+
Influenza A/California/7/2009(H1N1)pdm09 virus	-/+	Influenza A/common magpie/Hong Kong/645/2006 (H5N1) virus	-/+	Influenza B/Texas/3073/2013 virus	-/+
Influenza A/Dominican Republic /7293/2013 (H1N1)pdm09 virus	-/+	Influenza A/duck/Hunan/795/2002 (H5N1) virus	-/+	Influenza B/Phuket/06/2011 virus	-/+
Influenza A/Massachussetts/15/2013 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/321/2007 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 virus	-/+
Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-/+	Influenza A/ Egypt /321/2007 x PR8-IDCDC-RG11 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 BX-41A virus	-/+
Influenza A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1)	-/+	Influenza A/ Egypt /3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1) virus	-/+	*Legionella bozemanii*	-
Influenza A/New Caledonia/20/99(H1N1) virus	-/+	Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 virus	-/+	*Legionella dumoffii*	-
Influenza A/New York/18/2009 (H1N1)pdm09 virus	-/+	Influenza A/Hong Kong/213/2003 (H5N1) virus	-/+	*Legionella longbeachae*	-
Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-/+	Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus	-/+	*Legionella micdadei*	-

Testiranje unakrsne reaktivnosti					
Influenza A/Sydney/134/2018 (H1N1)pdm09 virus	-/+	Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1) virus	-/+	*Legionella pneumophila*	-
Influenza A/Victoria/2040/2018 (H1N1)pdm09 virus	-/+	Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1) virus	-/+	Ljudski metapneumovirus A i B	-
Influenza A/PR/8/34 (H1N1) virus	-/+	Influenza A/Vietnam/1194/2004 (H5N1) virus	-/+	*Moraxella catarrhalis*	-
Influenza A/Brisbane/117/2018 (H3N2) virus	-/+	Influenza A/Vietnam/1194/2004 x PR8-IDCDC-RG11 (H5N1) virus	-/+	*Mycoplasma pneumoniae*	-
Influenza A/Brisbane/1028/2017 (H3N2) virus	-/+	Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1) virus	-/+	*Mycobacterium tuberculosis* koja nije otporna na rifampicin	-
Influenza A/Fujian/411/2002 (H3N2) virus	-/+	Influenza A/Whooper Swan/R65/2006 (H5N1) virus	-/+	Virusi ljudske parainfluence tipa 1, 2, 3 i 4	-
Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2) virus	-/+	Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus	-/+	*Pneumocytis jirovecii* tip A1 i g885652	-
Influenza A/Hong Kong/4801/2014 (H3N2) virus	-/+	Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3) virus	-/+	Ljudski rinovirus tip C	-
Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-/+	Influenza A/Duck/Lao/XBY004/2014 (H5N6) (Clade 2.3.4.4) virus	-/+	*Staphylococcus aureus* podsoj *aureus*	-
Influenza A/Indiana/8/2011 (H3N2)v virus	-/+	Influenza A/DE-SH/Reiherente/AR8444/ 2013 (H5N8) virus	-/+	*Staphylococcus epidermidis*	-
Influenza A/Indiana/10/2011 (H3N2)v virus	-/+	Influenza A/Turkey/Germany/R2485-86/2014 (H5N8) virus	-/+	*Streptococcus pneumoniae* Z022	-
Influenza A/Kansas/14/2017 (H3N2) virus	-/+	Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2) virus	-/+	*Streptococcus pyogenes*	-
Influenza A/Kansas/14/2017, NYMC X-327 (H3N2) virus	-/+	Influenza A/Mallard/Netherlands/2/2009 (H7N7) virus	-/+	*Streptococcus salivarius*	-
Influenza A/Kumamoto/102/2002 (H3N2) virus	-/+	Influenza A/Mallard/Nizozemska/12/2000 (H7N7) - IBCDC-1 virus	-/+	Respiratorni sincicijski virus (RSV) A i B (soj CH93(18)-18)	-/+
Influenza A/Minnesota/11/2010 (H3N2)v virus	-/+	Influenza A/Anhui/1/2013 (H7N9) virus	-/+	Ljudski respiratorni sincicijski virus soj Long	-/+
Influenza A/Minnesota/11/2010 X203 (H3N2)v virus	-/+	Influenza A/Guangdong/17SF003/2016 (H7N9) virus	-/+		

Tablica 14. Referentni patogeni mikroorganizmi korišteni u ovom ispitivanju.

12.4. Analitička reaktivnost

Reaktivnost VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System za **SARS-CoV-2** procijenjen je naspram RNK iz ljudskog soja 2019-nCoV BetaCoV/Germany/BavPat1/2020 p.1, ljudski soj 2019-nCoV 2019-nCoV/Italy-INMI1, SARS-CoV-2 strain 2019nCoV/USA-WA1/2020, sintetska RNK kontrolira dvije varijante virusa

SARS-CoV-2: MT007544.1 (SARS-CoV2 izolat Australia/VIC01/2020) i MN908947.3 (SARS-CoV-2 izolat Wuhan-Hu-1), i pokazala je pozitivne rezultate.

Reaktivnost kompleta za detekciju u stvarnom vremenu VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System za **Influenza A** procijenjen je prema RNK ekstrahirane iz sljedećih sojeva: Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09 virus, influenza A/California/7/2009(H1N1)pdm09 virus, Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09 virus, influenza A/Massachusetts/15/2013 (H1N1)pdm09 virus, Influenza A/Michigan/45/2015 (H1N1)pdm09 virus, Influenza A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1), Influenza A/New Caledonia/20/99(H1N1) virus, Influenza A/New York/18/2009 (H1N1)pdm09 virus, Influenza A/Singapore/GP1908/2015 virus, IVR-180 (H1N1)pdm09 virus, Influenza A/Sydney/134/2018 (H1N1)pdm09 virus, Influenza A/Victoria/2040/2018 (H1N1)pdm09 virus, Influenza A/PR/8/34 (H1N1) virus, Influenza A/Brisbane/117/2018 (H3N2) virus, Influenza A/Brisbane/1028/2017 (H3N2) virus, influenza A/Fujian/411/2002 (H3N2) virus, influenza A/Hiroshima//52/2005 (IVR-142) (H3N2) virus, influenza A/Hong Kong/4801/2014 (H3N2) virus, Influenza A/Hong Kong/4801/2014 NYMC X-263B (H3N2) virus, influenza A/Indiana/8/2011 (H3N2)v virus, Influenza A/Indiana/10/2011 (H3N2)v virus, Influenza A/Kansas/14/2017 (H3N2) virus, influenza A/Kansas/14/2017, NYMC X-327 (H3N2) virus, Influenza A/Kumamoto/102/2002 (H3N2) virus, Influenza A/Minnesota/11/2010 (H3N2)v virus, Influenza A/Minnesota/11/2010 X203 (H3N2)v virus, Influenza A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a), influenza A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a), Influenza A/Newcastle/607/2019 (H3N2) virus, Influenza A/New York/39/2012 (H3N2) virus, Influenza A/Ohio/2/2012 (H3N2) virus, Influenza A/Perth/1001/2018 (H3N2) virus, Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2) virus, influenza A/South Australia/55/2014 (H3N2) virus, Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus, influenza A/Switzerland/9715293/2013 (H3N2) virus, Influenza A/Texas/50/2012 (H3N2) virus, Influenza A/Thüringen/5/2017 (H3N2) virus (Clade 3C2a.1), influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C) virus, influenza A/Victoria/210/2009(H3N2) virus, Influenza A/Victoria/361/2011 (H3N2) virus, influenza A/Victoria/361/2011 IVR-165 (H3N2) virus, influenza A/Anhui/01/2005 (H5N1) virus, Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1) virus, influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1) virus, Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1) virus, influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus, influenza A/chicken/Yunnan/1251/2003 (H5N1) virus, influenza A/common magpie/Hong Kong/645/2006 (H5N1) virus, influenza A/duck/Hunan/795/2002 (H5N1) virus, influenza A/Egypt/321/2007 (H5N1) virus, Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1) virus, influenza A/ Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1) virus, Influenza A/ Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 virus, Influenza A/Hong Kong/213/2003 (H5N1) virus, Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus, Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1) virus, Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1) virus, Influenza A/Vietnam/1194/2004 (H5N1) virus, Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1) virus, Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1) virus, Influenza A/Whooper Swan/R65/2006 (H5N1) virus, Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus, Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3) virus, Influenza A/Duck/Lao/XBY004/2014 (H5N6) virus (Clade 2.3.4.4), Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus, Influenza A/Turkey/Germany/R2485-86/2014 (H5N8) virus, Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2) virus, Influenza A/Mallard/Neherlands/2/2009 (H7N7) virus, influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1 virus, Influenza A/Anhui/1/2013 (H7N9) virus, Influenza A/Guangdong/17SF003/2016 (H7N9) virus, influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2) virus, influenza A/chicken/Myanmar/433/2016 (H9N2) virus, Influenza A/Hong Kong/1073/99 (H9N2) virus, Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26 virus, pokazujući pozitivne rezultate.

Reaktivnost kompleta za detekciju u stvarnom vremenu VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System za **Influenza B** procijenjen je prema RNK ekstrahirane iz sljedećih sojeva: Influenza B/Brisbane/60/2008 virus, Influenza B/Colorado/6/2017 virus, Influenza B/Malaysia/2506/2004 virus, Influenza B/Maryland/15/2016 virus, Influenza B/Netherlands/207/06 virus, Influenza B/Netherlands/2518/2016 (clade 1A) virus, Influenza B/Nevada/3/2011 virus, Influenza B/New Jersey/1/2012 virus, Influenza B/Texas/02/2013 virus , Influenza B/Townsville/8/2016 virus (**B/Victoria linija**); Influenza B/Canberra/11/2016 virus, Influenza B/Florida/4/2006 virus, Influenza B/Florida/07/2004 virus, Influenza B/Guangdong/120/2000 virus, Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus, Influenza B/Jiangsu/10/2003 virus, Influenza B/Massachusetts/2/2012 virus, Influenza B/Netherlands/365/2016 (clade 3) virus, Influenza B/Phuket/3073/2013 virus, Influenza B/Texas/06/2011 virus, Influenza B/Wisconsin/1/2010 virus, Influenza B/Wisconsin/1/2010 BX-41A virus (**B/Yamagata linija**), pokazujući pozitivan rezultat.

Reaktivnosti kompleta VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System za **RSV** potvrđena je naspram RNK ekstrahirane iz RSV A i B (soj CH93(18)-18) te soja humanog respiratornog sincicijskog virusa Long, pokazujući pozitivne rezultate.

13. 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 September 2020.
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 September 2020.
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 September 2020.

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 September 2020.
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 September 2020.
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 September 2020.
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 September 2020.
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 September 2020.
21. G. Neumann *et al*. Transmission of Influenza A viruses. *Virology* 2015; 234-246.
22. Y. Yang et al. Simultaneous typing and HA/NA subtyping of influenza A and B viruses including the pandemic influenza A/H1N1 2009 by multiplex real-time RT-PCR. *Journal of Virological Methods* 2010; 167(1): 37-44.
23. R.L. Kuo *et al*. Influenza A/B virus detection and influenza A virus subtyping with emphasis on the novel H7N9 virus by using multiplex real-time RT-PCR. *Journal of Virological Methods* 2014; 208:41-46.
24. World Health Organization. WHO information for molecular diagnosis of influenza virus—update. Available from: https://www.who.int/influenza/gisrs_laboratory/molecular_diagnosis/en/ . Accessed September 2020.
25. S. Subhash Bawage *et al*. Recent Advances in Diagnosis, Prevention, and Treatment of Human Respiratory Syncytial Virus. *Advances in Virology* 2013.
26. French, *et al*. Risk of nosocomial respiratory syncytial virus infection and effectiveness of control measures to prevent transmission events: a systematic review. *Influenza and Other Respiratory Viruses* 2016.
27. X. Yu *et al*. Human respiratory syncytial virus in children with lower respiratory tract infections or influenza-like illness and its co-infection characteristics with viruses and atypical bacteria in Hangzhou, China. *Journal of Clinical Virology* 2015; 69:1-6.

28. N. Mazur *et al*. Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. *The Lancet Respiratory Medicine* 2015; 3: 888-900.
29. F. de-Paris *et al*. Optimization of one-step duplex real-time RT-PCR for detection of influenza and respiratory syncytial virus in nasopharyngeal aspirates. *Journal of Virological Methods* 2012; 186(1-2): 189-192.
30. A. Hu *et al*. Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR. *Journal of Clinical Microbiology* 2003; 41(1): 149-154.
31. M. Hindiyeh *et al*. Evaluation of Simplexa Flu A/B & RSV for direct detection of influenza viruses (A and B) and respiratory syncytial virus in patient respiratory samples. *Journal of Clinical Microbiology* 2013; 51(7): 2421-2424.

14. 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

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