VIASURE

Real Time PCR Detection Kit

Respiratory Virus Mix I

for BD MAX™ System

CE IVD

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

PRODUCT / PRODUKT	REFERENCE / REFERENCE
VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System	444219 / VS-SFR124

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

NOTE: Instructions for use (IFU) are included into the kit in English/Spanish version / Brugsanvisningen er inkluderet i sættet i en engelsk/spansk version.

EN For download IFUs from other languages, please enter in **certest.es/viasure/labeling**. Once you be there, follow the instructions for access to the language that you need. If you need additional information, please contact: viasure@certest.es.

ES Para descargar las IFUS en otros idiomas, por favor, entre en **certest.es/viasure/labeling.** Una vez esté allí, siga las instrucciones para acceder al idioma que necesite. Si necesita información adicional, contacte: viasure@certest.es.

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FR Pour télécharger l'IFUS dans d'autres langues, veuillez vous rendre sur **certest.es/viasure/labeling.** Une fois sur place, suivez les instructions pour accéder à la langue dont vous avez besoin. Si vous avez besoin d'informations supplémentaires, contactez: viasure@certest.es.

IT Per scaricare l'IFUS in altre lingue, vai su **certest.es/viasure/labeling**. Una volta che sei lì, segui le istruzioni per accedere alla lingua di cui hai bisogno. Se hai bisogno di ulteriori informazioni, contatta: viasure@certest.es.

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PT Para baixar o IFUS em outros idiomas europeus, acesse **certest.es/viasure/labeling**. Uma vez lá, siga as instruções para acessar o idioma que você precisa. Se você precisar de informações adicionais, entre em contato: viasure@certest.es.

SV För att ladda ner IFUS från andra språk, vänligen gå in på **certest.es/viasure/labeling**. När du är där följer du instruktionerna för att få tillgång till det språk du behöver. Om du behöver ytterligare information kan du kontakta: viasure@certest.es.

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Contact viasure@certest.es if your language is not on the list / Kontakt viasure@certest.es, hvis dit sprog ikke er med på listen.

Note: The user should notify the manufacturer and the competent authority of the Member State in which he is established as a user and/or patient of any serious incident related to the product.

Bemærk: Brugeren skal underrette fabrikanten og den kompetente myndighed i landet, hvor den pågældende er bosiddende som bruger og/eller patient, om enhver alvorlig hændelse i forbindelse med produktet.

Content

Indhold

ENGLISH

1. Intended use

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System is an automated real-time RT-PCR test designed for the qualitative detection of RNA from SARS-CoV-2, Influenza B, Influenza A and RSV (types A and B) in respiratory samples (nasopharyngeal swabs) from patients suspected of respiratory infection by their healthcare professional (HCP). This test is intended to be used as an aid in the identification of SARS-CoV-2, Influenza B, Influenza A and RSV (types A and B) infection in combination with patient's clinical signs and symptoms and epidemiological risk factors. The assay uses the BD MAX™ System for automated extraction of RNA and subsequent real-time RT-PCR, employing the reagents provided combined with universal reagents and disposables for the BD MAX™ System. RNA is extracted from specimens, and complementary DNA (cDNA) is synthetized and amplified using RT-PCR and detected using fluorescent reporter dye probes specific for SARS-CoV-2, Influenza B, Influenza A and RSV (types A and B).

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 *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System is designed for the qualitative detection of RNA from SARS-CoV-2, Influenza B, Influenza A and RSV (types A and B) in nasopharyngeal swabs. The detection is done in one step real-time RT-PCR format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction tube. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase, which is followed by the amplification of a conserved region of *N* and *ORF1ab* genes of SARS-CoV-2, *M1* gene of Influenza B, *M1* gene of Influenza A and *N* gene of RSV (types A and B) using specific primers and a fluorescent-labelled probe.

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

Target	Channel	Gene
SARS-CoV-2	475/520	*N* and *ORF1ab* gene
Influenza B	530/565	*Matrix* gene (*M1*)
Influenza A	585/630	*Matrix* gene (*M1*)
RSV (A/B)	630/665	*N* gene
Endogenous Internal Control (EIC)	680/715	Human *RNase P* gene

Table 1. Target, channel and genes.

4. Reagents provided

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System includes the following materials and reagents detailed in Table 2:

Reagent/Material	Description	Barcode	Amount
Respiratory Virus Mix I reaction tube	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and endogenous internal control in stabilized format	1K foil	2 pouches of 12 transparent tubes
Rehydration Buffer tube	Solution to reconstitute the stabilized product	11 foil	1 pouch of 24 transparent tubes

Table 2. Reagents and materials provided in VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System with Cat. N°. VS-SFR124 (444219).

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 *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System.

- Real-time PCR instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442828 or 442827).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).
- Nuclease-free water.
- Filter tips.
- Powder-free disposable gloves.

6. Transport and storage conditions

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

7. Precautions for users

- The product is intended for use by professional users only, such as laboratory or health care professionals and technicians, trained in molecular biological techniques.
- For *in vitro* diagnostic use.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective box is open or broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Protect reagents from humidity. Prolonged exposure to humidity may affect product performance.
- Keep components away from light.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, any additional reagents required for testing and the BD MAX™ System are not contaminated. Always avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges (BD MAX™ PCR Cartridge).
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridge are designed to prevent contamination.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles and mask. Do not eat, drink, smoke or apply cosmetic products in the working area. Wash your hands after finishing the test.
- Samples must be treated as potentially infectious and/or biohazardous, as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national safety regulations. Take necessary precautions during the collection, transport, storage, handling, and disposal of samples.

- Samples and reagents must be handled in a biological safety cabinet. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Dispose of waste in compliance with local and state regulations.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- In accordance with Regulation (EC) No 1907/2006 (REACH), VIASURE Real Time PCR Detection Kits do not require Material Safety Data Sheets on account of their classification as non-hazardous to health and the environment, because they do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) No 1272/2008 (CLP), or which are in concentrations higher than the value established in the mentioned regulation for their declaration.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Test procedure

8.1. Sample collection, storage and transport

The VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System has been tested on nasopharyngeal swabs collected in sterile Vircell® transport medium or in sterile Virus transport and preservation medium (Biocomma®), and in Universal Transport Medium, depending on the sample. Other types of samples must be validated by the user.

Collection, storage and transport of specimens should be maintained per the conditions validated by the user. Overall, respiratory samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type) and processed as soon as possible to guarantee the quality of the test. The specimens should be transported at 2 to 8°C for up to 72 hours, following the local and national regulations for the transport of pathogen material. For long term transport (more than 72 hours), we recommend shipping at ≤-20°C or lower. It is recommended to use fresh specimens for the test. The samples can be stored at 2 to 8°C for up to 72 hours or frozen at -20°C or ideally at -70°C for conservation. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

The clinical samples must be collected, transported and stored according to appropriate laboratory guidelines. For details, refer to the CDC guideline (Specimen collection guidelines. Website https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf), the IDSA guideline (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94) and García-Lechuz Moya, J.M., González López, J.J., Orta Mira, N., Sánchez Romero, M.I. (2017). Recogida, transporte y procesamiento general de las muestras en el Laboratorio de Microbiología. 2017. 1b. Sánchez Romero, M.I., (coordinadora). Procedimientos en Microbiología Clínica. Cercenado Mansilla, E., Cantón Moreno, R., (editores). *Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC)*.

8.2. Sample preparation and DNA 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 400-750 µL of nasopharyngeal samples into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

8.3. PCR protocol

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

8.3.1. Creating PCR test program for VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System

Note: If you have already created the test for the VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System, you can skip step 8.3.1 and go directly to 8.3.2.

1) On the "Run" screen of the BD MAX™ System, select the "Test Editor" tab.
2) Click the "Create" button.
3) In the Basic Information tab, within the "Test Name" window, name your test: i.e. VIASURE *Respiratory Virus Mix I.*
4) In the "Extraction Type" drop down menu, select "ExK TNA-3".
5) In the "Master Mix Format" drop down menu, choose "Type 5".
    a. Note: Product may be used in combination with an additional VIASURE for BD MAX test, then select "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)".
6) In the "Sample extraction parameters" select "User defined" and adjust sample volume to 950 µL.
7) In the "Ct Calculation" select "Call Ct at Threshold Crossing".
8) If running software version 5.00 or higher and have barcoded foil snap-in tubes, in the "Custom Barcodes" select the following configuration:
    a. Snap-In 2 Barcode: 1K (concerning *Respiratory Virus Mix I* reaction tube).
    b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube).
    c. Snap-In 4 Barcode: another reaction tube (different foil) if you choose the format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Section 8.3.1).
9) In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 3).
    a. Note: Product may be used in combination with an additional VIASURE for BD MAX™ test, PCR Settings and Test Steps should be completed for snap 2 (green) and snap 4 (blue) positions.

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	SARS-CoV-2	80	150	0	40
530/565 (HEX)	Influenza B	80	150	0	40
585/630 (ROX)	Influenza A	80	150	0	40
630/665 (Cy5)	RSV (A/B)	80	150	0	40
680/715 (Cy5.5)	EIC	80	150	0	35

Table 3. PCR settings.

Note: It is recommended to set the minimum threshold values listed above for each channel as a starting point, but the final settings must be determined by the end-user during the result interpretation, in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments may vary due to different signal intensities.

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

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	4.0	0.0	0.0	0.0
	530/565	1.0	-	0.0	0.0	0.0
	585/630	0.0	0.0	-	1.0	0.0
	630/665	0.0	0.0	3.0	-	18.0
	680/715	0.0	0.0	0.0	1.5	-

Table 4. Spectral cross-talk parameters.

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

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

Table 5. PCR protocol.

12) Click the "Save Test" button.

8.3.2. BD MAX™ Rack set up

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

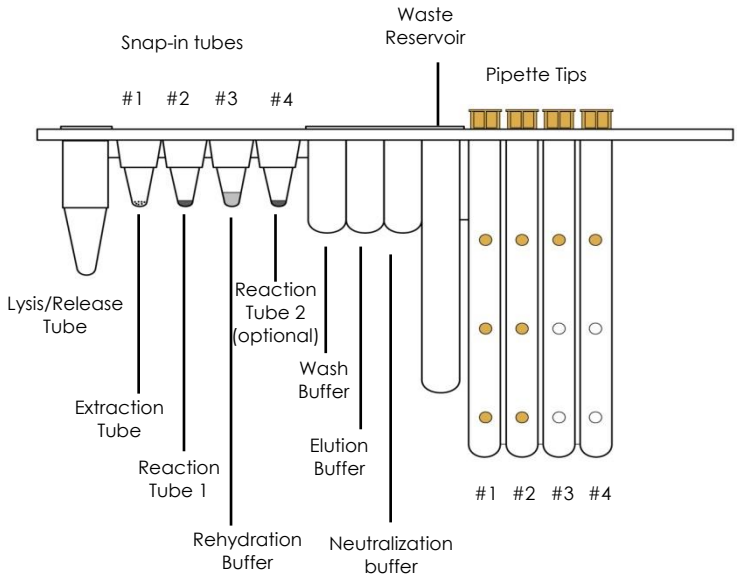
b. In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.

i. Note: If you choose the format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Section 8.3.1), determine and separate the appropriate number of additional VIASURE reaction tubes (different foil) and snap into their corresponding positions in the strip (Snap position 4, blue color coding on the rack. See Figure 1). Remove excess air, and close aluminum pouches with the zip seal.

4) Remove the required number of Rehydration Buffer tubes (11 foil) and snap into their corresponding positions in the strip (Snap position 3, non-color coding on the rack. See Figure 1). Remove excess air and close the pouch with the zip seal.

a. In order to ensure a correct transfer, please make sure that the liquid is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the buffer is at the bottom of the tube.

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



8.3.3. BD MAX™ Instrument set up

1) Select the "Work List" tab on the "Run" screen of the BD MAX™ System software v4.50A or higher.
2) In the "Test" drop down menu, select VIASURE *Respiratory Virus Mix I* (if not already created see Section 8.3.1).
3) Select the appropriate kit lot number (found on the outer box of extraction kit used) from the pull-down menu (optional).
4) Enter the Sample Buffer Tube identification number into the Sample tube window of the Work List, either by scanning the barcode with the scanner or by manual entry.
5) Fill the Specimen/Patient ID and/or Accession window of the Work List and click the "Save" button. Continue until all Sample Buffer Tubes are entered. Ensure that the specimen/patient ID and the Sample Buffer Tubes are accurately matched.
6) Place the prepared Sample Buffer Tube into the BD MAX™ Rack(s).

7) Load the rack(s) into the BD MAX™ System (Rack A is positioned on the left side of the BD MAX™ System and Rack B on the right side).
8) Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System.
9) Close the BD MAX™ System door.
10) Click "Start Run" to begin the procedure.

8.3.4. BD MAX™ report

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

9. Result interpretation

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

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

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

- Ct value of -1 indicates that no amplification process has occurred that meets the setting criteria.

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

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

Results should be read and analyzed using the following table:

SARS-CoV-2 (475/520)	Flu B (530/565)	Flu A (585/630)	RSV (A/B) (630/655)	Endogenous Internal Control (680/715)	Interpretation
+	+	+	+	+/-[1]	**SARS-CoV-2, Flu B, Flu A and RSV (A/B) RNA detected[1]**
-	+	+	+	+/-[1]	**Flu B, Flu A, RSV (A/B) RNA detected and SARS-CoV-2 RNA not detected[1]**
+	-	+	+	+/-[1]	**SARS-CoV-2, Flu A, RSV (A/B) RNA detected and Flu B RNA not detected[1]**
+	+	-	+	+/-[1]	**SARS-CoV-2, Flu B, RSV (A/B) RNA detected and Flu A RNA not detected[1]**
+	+	+	-	+/-[1]	**SARS-CoV-2, Flu B, Flu A RNA detected and RSV (A/B) RNA not detected[1]**
+	+	-	-	+/-[1]	**SARS-CoV-2, Flu B RNA detected and Flu A, RSV (A/B) RNA not detected[1]**
+	-	+	-	+/-[1]	**SARS-CoV-2, Flu A RNA detected and Flu B, RSV (A/B) RNA not detected[1]**
+	-	-	+	+/-[1]	**SARS-CoV-2, RSV (A/B) RNA detected and Flu B, Flu A RNA not detected[1]**
-	+	+	-	+/-[1]	**Flu B, Flu A RNA detected and SARS-CoV-2, RSV (A/B) RNA not detected[1]**
-	+	-	+	+/-[1]	**Flu B, RSV (A/B) RNA detected and SARS-CoV-2, Flu A RNA not detected[1]**
-	-	+	+	+/-[1]	**Flu A, RSV (A/B) RNA detected and SARS-CoV-2, Flu B RNA not detected[1]**
+	-	-	-	+/-[1]	**SARS-CoV-2 RNA detected[1]**
-	+	-	-	+/-[1]	**Influenza B RNA detected[1]**
-	-	+	-	+/-[1]	**Influenza A RNA detected[1]**
-	-	-	+	+/-[1]	**RSV RNA detected[1]**
-	-	-	-	+[2]	**SARS-CoV-2, Flu B, Flu A and RSV (A/B) 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	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	INC	**Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run.**

Table 6. Sample interpretation.
+: Amplification occurred.
-: No amplification occurred.

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

2 A sample is considered negative if the sample shows no amplification signal in the detection system, but the endogenous internal control is positive (Ct less than 35). 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 following the indications below.

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 PCR steps and review the parameters; and to check the sigmoid shape of the curve and the intensity of fluorescence.

NOTE: New samples may be tested in the same run with repeat samples.

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 swabs.
- 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, Influenza B, Influenza A or RSV (A/B), 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 the *N* and *ORF1ab* genes of SARS-CoV-2, *M1* gene of Influenza B, *M1* gene of Influenza A and *N* gene of RSV (types A and B) used in VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System do not show significant combined homologies with the human genome, human microflora, or other respiratory microorganisms, 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 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, Influenza B, Influenza A or RSV (A/B) strains.
    - Organism levels in the specimen below the limit of detection or cutoff for the assay.

- The presence of qPCR inhibitors or other types of interfering substances. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs used to prevent COVID-19, gripe and RSV or used during the treatment of the infection have not been evaluated.
- Failure to follow instructions for use and the assay procedure.

- Some samples may fail to exhibit *RNase P* amplification curves due to low human cell numbers in the original clinical sample. A negative IC signal does not preclude the presence of SARS-CoV-2, Influenza B, Influenza A or RSV (A/B) in a clinical specimen.
- A positive test result does not necessarily indicate the presence of viable virus and does not imply that these virus are infectious or are the causative agents for clinical symptoms. However, a positive result is indicative of the presence of target viral sequences.
- If diagnostic tests for other respiratory illnesses are negative and the patient's clinical presentation and epidemiological information suggest that SARS-CoV-2, Influenza B, Influenza A or RSV (A/B) infection is possible, then a false negative result should be considered, and a re-testing of the patient should be discussed.
- A negative result does not preclude the presence of target *RNA* in a clinical specimen. If clinical observations, patient history and epidemiological information suggest SARS-CoV-2, Influenza B, Influenza A or RSV (A/B) infection, re-testing increasing sample volume should be considered.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *Respiratory Virus Mix I 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 *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System contains an Endogenous Internal Control (EIC) in each reaction tube which confirms the correct performance of the technique.

12. Performance characteristics

12.1. Clinical sensitivity and specificity

The clinical performance of VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System was tested using clinical samples (nasopharyngeal swabs) from patients with clinical suspicion of SARS-CoV-2, Flu A/B and or RSV A/B infection. The results were as follows:

	Site	Sample type	Workflow	Target
1	CerTest Biotec S.L. in collaboration with the Biobank of the Sistema de Salud de Aragón (BSSA) and the Microbiology and Parasitology Department of Hospital Universitario Central de Asturias	Nasopharyngeal swabs	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	SARS-CoV-2
				Influenza B
				Influenza A
				RSV (A/B)

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

True positive and negative values, false positive and negative values, sensitivity, and specificity for VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System were calculated in relation to each comparator assay as shown in the following table:

Site	Comparator assay	Target	TP	TN	FP	FN	Sensitivity	Specificity
1	TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific) or VIASURE *SARS-CoV-2* Real time PCR Detection Kit, + subsequent whole genome sequencing	SARS-CoV-2	127	625	1	3	0.977 (0.934-0.995)	0.998 (0.991-1)
	Cobas® Influenza A/B & RSV Nucleic acid test for use on the cobas® Liat® System (cobas® Influenza A/B & RSV)	Influenza B	18	738	0	0	1 (0.815-1)	1 (0.995-1)
		Influenza A	49	704	1	2	0.961 (0.865 – 0.995)	0.999 (0.992-1)
		RSV (A/B)	50	706	0	0	1 (0.929-1)	1 (0.995-1)

Table 8. True positive and negative values, false positive and negative values, sensitivity, specificity for VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System.

Result show agreement to detect SARS-CoV-2, Influenza B, Influenza A and RSV (A/B) using VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytical sensitivity

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System detection limit (LoD) results on nasopharyngeal samples with a positive rate of ≥ 95% are as follows:

a) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of 5.01 IU (International Units)/µl for SARS-CoV-2.
b) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of 1.8 x 10^2 $CEID_{50}$ (Median Chicken Embryo Infectious Dose)/ml for Influenza B.
c) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of $10^{-0.5}$ $TCID_{50}$ (Median Tissue Culture Infectious Dose) /ml for Influenza A.
d) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of 4 genome copies/µl for RSVA and RSVB.

Note: The detection limit was calculated using a sample volume of 400 µl.

Examples of the amplification plots resulting from running an assay on the BD MAX™ System are shown below

Figure 2. Dilution series of SARS-CoV-2 (5x10^5-5x10^0 copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).

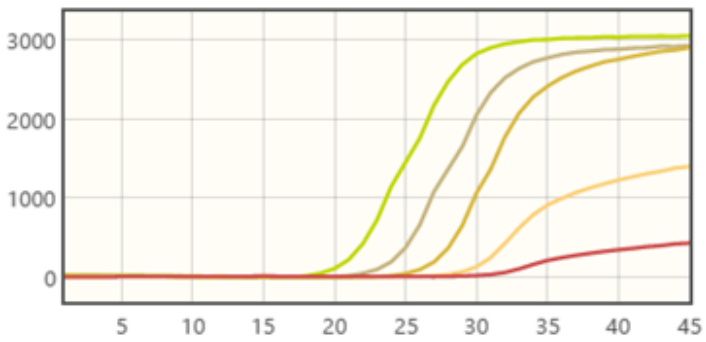


Figure 3. Dilution series of Influenza B (5x10^5-5x10^0 copies per reaction) template run on the BD MAX™ System (530/565 (HEX) channel).

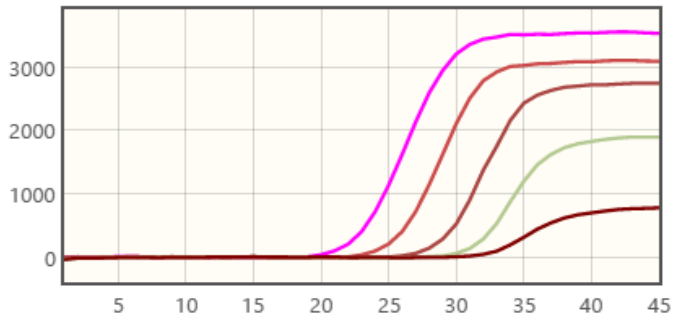


Figure 4. Dilution series of Influenza A (5x10^5-5x10^0 copies per reaction) template run on the BD MAX™ System (585/630 (ROX) channel).

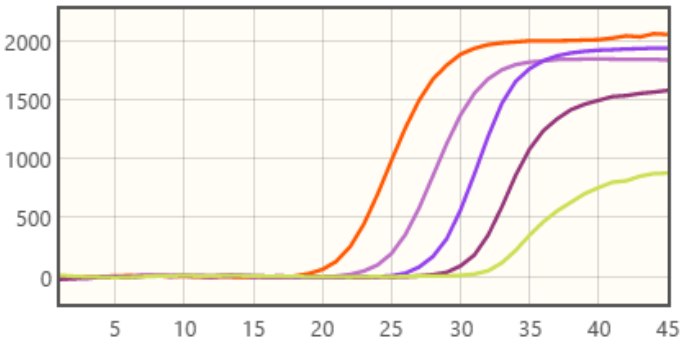


Figure 5. Dilution series of RSVA (5x10^5-5x10^0 genome copies per reaction) template run on the BD MAX™ System (630/665 (CY5) channel).

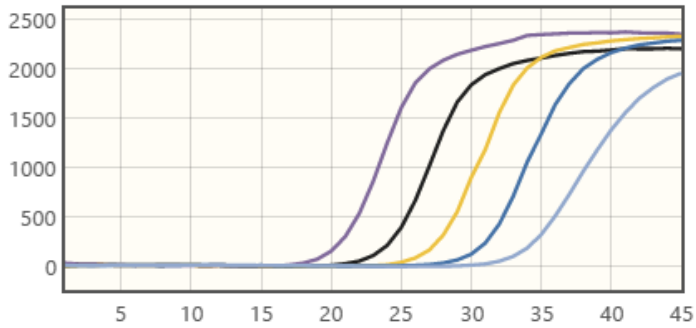
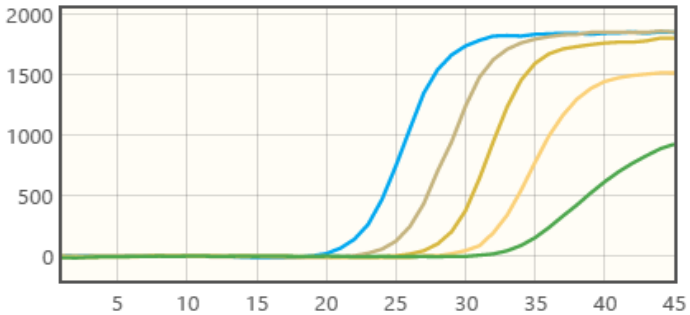


Figure 6. Dilution series of RSVB (5x10^5-5x10^0 genome copies per reaction) template run on the BD MAX™ System (630/665 (CY5) channel).



12.3. Analytical specificity

The specificity of the *Respiratory Virus Mix I* assay was confirmed by testing a panel consisting of different microorganisms representing the most common respiratory pathogens. No cross-reactivity was detected between any of the following microorganisms tested:

Cross-reactivity testing					
Human Adenovirus types 1-5, 8, 15, 31, 40 and 41	-	Enterovirus Coxsackievirus A24, A9 and B3	-	Mycoplasma pneumoniae	-
Bocavirus	-	Enterovirus Echovirus 30	-	Mycobacterium tuberculosis	-
Bordetella bronchiseptica	-	Enterovirus 68, 71	-	Human parainfluenza 1, 2, 3 and 4 viruses	-
Bordetella holmesii	-	Haemophilus influenzae MinnA	-	Pneumocytis jirovecii Type A1 and g885652	-
Bordetella parapertussis	-	Legionella bozemanii	-	Human rhinovirus	-
Bordetella pertussis	-	Legionella dumoffii	-	SARS Coronavirus Strain Frankfurt 1	-
Chlamydia caviae	-	Legionella longbeachae	-	Staphylococcus aureus	-
Chlamydia psittaci genotype A and C	-	Legionella micdadei	-	Staphylococcus epidermidis	-
Chlamydophila pneumoniae CM-1	-	Legionella pneumophila	-	Streptococcus pneumoniae	-
Human coronavirus 229E, OC43, NL63 and HKU1	-	Human metapneumovirus A and B	-	Streptococcus pyogenes	-
MERS Coronavirus	-	Moraxella catarrhalis	-	Streptococcus salivarius	-

Table 9. Reference pathogenic microorganisms used in this study.

12.4. Analytical reactivity

The reactivity of VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **SARS-CoV-2** was evaluated against RNA extracted from Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1, Human 2019-nCoV strain 2019-nCoV/Italy-INMI1, synthetic RNA controls for MT007544.1 variant (SARSCoV2 isolate Australia/VIC01/2020), MN908947.3 variant (SARS-CoV-2 isolate Wuhan-Hu-1), alpha variant (B.1.1.7 England/MILK-9E05B3/2020), Beta variant (B.1.351 South Africa/KRISP-EC-K005299/2020), Gamma variant (P.1 Japan (Brazil) /IC-0564/2021) and Kappa variant (B.1.617.1 India/CT-ILSGS00361/2021), and heat inactivated SARSCoV-2 strain 2019nCoV/USAWA1/2020 (ATCC® VR1986HK™), and irradiated cell lysate from 2019-nCoV/USA-WA1/2020, and lyophilized cell lysates from BetaCoV/Berlin/ChVir1670/2020_IsolatBER, BetaCoV/Munich/ChVir984/2020 and BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER, showing positive results.

The reactivity of VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **Influenza B** was evaluated against RNA extracted from the following strains: B/Phuket/3073/2013 virus, B/Brisbane/60/2008 virus, Influenza B/Florida/04/06 virus, B/Pennsylvania/7/2007 (Yamagata Lineage), B/Santiago/4364/2007 (Yamagata Lineage) virus, B/Brisbane/3/2007 (Yamagata Lineage) virus, B/Pennsylvania/5/2007 (Victoria Lineage), B/Victoria/304/2006 (Victoria Lineage) virus, B/Bangladesh/3333/2007 (Yamagata Lineage) virus, showing positive results.

The reactivity of VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **Influenza A** was evaluated against RNA extracted from the following strains: A/Switzerland/9715293/2013 (H3N2) virus, A/Thüringen/5/2017 (H3N2) virus, A/DE-SH/Reiherente/AR8444/ 2016(H5N8) virus, A/Anhui/1/2013 (H7N9) virus,

A/Michigan/45/2015 (H1N1 pdm09) virus, A/California/7/2009 (H1N1) virus, A/California/7/2009 (H1N1pdm09) virus, A/South Australia/55/2014 virus, Switzerland/9715293/2013 (H3N2) IVR-175 virus, A/Singapore/GP1908/2015 IVR-180 virus, A/Hong Kong/4801/2014 NYMC X-263B virus, Influenza A/New Caledonia/20/99 (H1N1) virus, A/Brisbane/59/2007 (H1N1) virus, A/South Dakota/6/2007 (H1N1) virus, A/Hawaii/31/2007 (H1N1) virus, A/Qatar/1123/2007 (H1N1) virus, A/Cambodia/0371/2007 (H1N1) virus , Influenza A Virus, A/Brisbane/10/2007 (H3N2) virus, Influenza A Virus, A/Taiwan/760/2007 (H3N2) virus, Influenza A Virus, A/Texas/71/2007 (H3N2) virus, A/Brisbane/10/2007 (H3N2) IVR-147 virus, A/Brisbane/59/2007 (H1N1) IVR-148 virus, A/South Dakota/6/2007 (H1N1) X-173 virus, A/California/07/2009 (H1N1)pdm09 virus, A/California/08/2009 (H1N1)pdm09 virus, A/New York/18/2009 (H1N1)pdm09 virus, A/Mexico/4108/2009 (H1N1)pdm09 virus, A/California/07/2009 (H1N1 pdm09) NYMC X-179A virus, A/Victoria/2570/2019 IVR-215 virus and A/Cambodia/e0826360/2020 IVR-224 virus, showing positive results.

The reactivity of VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **RSV** was evaluated against RNA extracted from Respiratory Syncytial Virus A (strain A-2) and Respiratory Syncytial Virus B (strain 9320), showing positive results.

DANSK

1. Anvendelsesformål

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System er en automatiseret real-time RT-PCR-test, der er designet til kvalitativ detektion af RNA fra SARS-CoV-2, Influenza B, Influenza A og RSV (type A og B) i respiratoriske prøver (nasofaryngeale prøver) fra patienter, som ifølge deres læge (HCP) har en mulig luftvejsinfektion. Testen er beregnet til at blive anvendt som en hjælp til identifikation af infektioner med SARS-CoV-2, Influenza B, Influenza A og RSV (type A og B) og skal sammenholdes med patientens kliniske tegn og symptomer og epidemiologiske risikofaktorer. Analysen anvender BD MAX™ System til automatisk ekstraktion af RNA og efterfølgende realtids-RT-PCR med anvendelse af de medfølgende reagenser kombineret med universelle reagenser og engangsartikler til BD MAX™ System. RNA ekstraheres fra prøverne, og komplementært DNA (cDNA) syntetiseres og amplificeres ved hjælp af RT-PCR og detekteres med fluorescensmærkede reporter-farveprober, der er specifikke for SARS-CoV-2, Influenza B, Influenza A og RSV (type A og B).

2. Oversigt og forklaring

Coronavirus er en kappeklædt, ikke-segmenteret virus med en positivt rettet RNA-streng, og den tilhører familien Coronaviridae. Seks coronavirusarter vides at forårsage sygdomme hos mennesker. Fire vira (229E, OC43, NL63 og HKU1) forårsager almindelige forkølelsessymptomer, og de to andre (svært akut respiratorisk syndrom coronavirus (SARS-CoV) og Mellemøstens respiratoriske syndrom coronavirus (MERS-CoV)) er zoonotiske og forårsager mere alvorlige komplikationer. SARS-CoV og MERS-CoV har forårsaget mere end 10.000 kumulative tilfælde i de seneste to årtier med en dødelighed på 34 % for MERS-CoV og 10 % for SARS-CoV.

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

Overførsel af SARS-CoV-2 fra menneske til menneske er blevet bekræftet, selv i inkubationsperioden uden symptomer, og virusset forårsager alvorlige luftvejslidelser, som ligner dem SARS-CoV frembragte. Selv om lungebetændelse er den hyppigste sygdom, har enkelte patienter udviklet svær lungebetændelse, lungeødem, akut åndedrætsbesvær eller multiorgansvigt og død. Centers of Disease Control and Prevention (CDC) mener, at symptomer på SARS-CoV-2 kan opstå så få som 2 dage eller så længe som 14 dage efter eksponering, hvor de mest almindelige er feber eller kulderystelser, hoste, træthed, appetitløshed, myalgi og dyspnø. Mindre almindelige symptomer er ondt i halsen, tilstoppet næse hovedpine, diarré, kvalme og opkastning. Tab af lugt (anosmi) eller tab af smag (ageusi) forud for forekomsten af luftvejssymptomer er også blevet rapporteret. Ældre voksne og personer, der har alvorlige underliggende medicinske tilstande som hjerte- eller lungesygdom eller diabetes, synes at have større risiko for at udvikle mere alvorlige komplikationer i forbindelse med COVID-19-sygdom.

CDC anbefaler prøver fra de øvre luftveje (nasofaryngeale (NP) og orofaryngeale (OP) podninger, nasal midt-turbinat podning, nasalpodning, nasofaryngeal skylning/aspirat eller nasalskylning/aspirat (NW), der primært indsamles af sundhedspersonale) og/eller prøver fra de nedre luftveje (sputum, endotrakealt aspirat eller bronchoalveolær skylning hos patienter med mere alvorlig luftvejssygdom) til identifikation af SARS-CoV-2 og andre luftvejsvira, såsom influenza og RSV.

Influenzavirus tilhører familien Orthomyxoviridae og er årsag til størstedelen af virusinfektioner i det nedre luftveje. Influenza A og B er en væsentlig årsag til sygelighed og dødelighed på verdensplan, og ældre og svækkede personer er særligt udsatte for at udvikle alvorlig sygdom og komplikationer såsom lungebetændelse. De fleste mennesker oplever nogle eller alle disse symptomer: feber eller feber/kulderystelser, hoste, ondt i halsen, tilstoppet næse og næseflåd, muskelsmerter, hovedpine og appetitløshed. Influenzavirusserne kan spredes fra person til person på to forskellige måder: gennem luften (store dråber og aerosoler fra nysen og hoste) og ved direkte eller indirekte kontakt.

Influenza A og B er et omsluttet, enkeltstrenget RNA-virus, der indeholder otte segmenterede strenge af genom-RNA, som typisk koder for 11 eller 12 virale proteiner. Viruskonvolutten, der stammer fra værtsplasmamembranen, består af en lipid-dobbeltlag indeholdende transmembranproteiner, som hæmagglutinin (HA) og neuraminidase (NA) og matrixproteiner M1 og M2. Influenza A-virus er yderligere klassificeret i undertyper baseret på antigeniciteten af deres "HA" og "NA" molekyler, mens Influenza B er opdelt i 2 antigenetisk og genetisk forskellige slægter, Victoria og Yamagata.

Human respiratorisk syncytialvirus A og B (RSV) tilhører familien Paramyxoviridae, og disse er de vigtigste virale agenser i luftvejsinfektioner. RSV er et kappeklædt, ikke-segmenteret, negativt, enkeltstrenget RNA-virus med lineært genom. Respiratorisk syncytialvirus er en almindelig bidragyder til luftvejsinfektioner og forårsager bronkitis, lungebetændelse, og kroniske obstruktive lungeinfektioner hos mennesker i alle aldre. Personerne oplever ofte nogle eller alle af disse symptomer: snue, lav grad af feber, hoste, ondt i halsen, hovedpine, og en hvæsende vejrtrækning. RSV overføres via store nasofaryngeale sekretionsdråber fra inficerede personer, tæt kontakt eller selvinokulering efter berøring af kontaminerede overflader.

Diagnose kan være problematisk, da en lang række patogener kan forårsage akutte luftvejsinfektioner med lignende kliniske symptomer. Realtids-PCR-analyser har vist sig at være et følsomme og specifikke diagnostiske værktøjer til påvisning af SARS-CoV-2-, influenza-A-, influenza-B- og RSV-vira.

3. Procedurens princip

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System er designet til kvalitativ detektion af RNA fra SARS-CoV-2, Influenza B, Influenza A og RSV (type A og B) fra nasofaryngeale podninger. Detektionen foretages i et-trins realtids-PCR-format, hvor revers transskription og efterfølgende forstærkning af den specifikke målsekvens finder sted i samme reaktionsrør. Det isolerede mål-RNA transkriberes under dannelse af komplementært DNA ved hjælp af revers transkriptase og efterfølges af amplifikation af en konserveret region i generne *N* og *ORF1ab* hos SARS-CoV-2, genet *M1* hos Influenza B, genet *M1* hos Influenza A og genet *N* hos RSV (type A og B) ved brug af specifikke primere og en fluorescensmærket probe.

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System er baseret på DNA-polymerasens 5´ exonucleaseaktivitet. Under DNA-forstærkningen spalter dette enzym proben, som er bundet til den komplementære DNA-sekvens, og adskiller quencher-farvestoffet fra reporteren. Denne reaktion genererer en stigning i det fluorescerende signal, som er proportional med mængden på målskabelonen. Denne fluorescens måles af BD MAX™ System.

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System indeholder alle komponenter i hvert rør, der er nødvendige for real-time PCR-analysen (specifikke primere/prober, dNTP'er, buffer, polymerase og

revers-transkriptase) i et stabiliseret format, såvel som en endogen intern kontrol til monitorering af ekstraktionsprocessen og/eller hæmning af polymeraseaktiviteten. Analysen anvender et humant housekeeping-gen som en endogen intern kontrol (EIC) (det humane *RNase P*-gen). Humane housekeeping-gener er involveret i basal cellevedligeholdelse og forventes derfor at være til stede i alle kerneholdige humane celler, og de opretholder relativt konstante ekspressionsniveauer.

Mål	Kanal	Gen
SARS-CoV-2	475/520	Generne *N* og *ORF1ab*
Influenza B	530/565	*Matrix*-genet (*M1*)
Influenza A	585/630	*Matrix*-genet (*M1*)
RSV (A/B)	630/665	*N*-genet
Endogen intern kontrol (EIC)	680/715	Humant *RNase P*-gen

Tabel 10. Mål, kanal og gener.

4. Leverede reagenser

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System omfatter de følgende materialer og reagenser, som er angivet i tabel 2:

Reagens/Materiale	Beskrivelse	Stregkode	Mængde
Respiratory Virus Mix I reaction tube	En blanding af enzymer, primer-prober, buffere, dNTP'er, stabilisatorer og endogene interne kontroller i stabiliseret format	1K folie	2 poser med 12 transparente rør
Rehydration Buffer tube	Opløsning til rekonstitution af det stabiliserede produkt	11 folie	1 pose med 24 transparente rør

Tabel 11. Reagenser og materialer, der leveres i VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System med kat. nr. VS-SFR124 (444219).

5. Reagenser og udstyr, der skal leveres af brugeren

Følgende angiver materialer og udstyr, der er nødvendige til brug men ikke er inkluderet i VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System.

- Realtids-PCR-instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442828 eller 442827).
- BD MAX™ PCR Cartridges (Ref: 437519).
- vortex.
- Mikropipetter (nøjagtighed mellem 2 og 1000 µl).
- Nukleasefrit vand.
- Filterspidser.
- Pulverfrie engangshandsker.

6. Transport- og opbevaringsforhold

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

7. Særlige forholdsregler for brugere

- Produktet er kun beregnet til brug af professionelle brugere, f.eks. laboratorie- eller sundhedspersonale og teknikere, der er uddannet i molekylærbiologiske teknikker.
- Til *in vitro*-diagnostisk brug.
- Brug ikke reagenser og/eller materialer, hvis udløbsdatoen er overskredet.
- Brug ikke sættet, hvis etiketten, der forsegler den ydre æske, er i stykker.
- Brug ikke reagenser, hvis beskyttelsesæsken er åben eller i stykker ved ankomsten.
- Brug ikke reagenser, hvis beskyttelsesposerne er åbne eller i stykker ved modtagelsen.
- Brug ikke reagenser, hvis tørremidlet ikke er til stede eller er i stykker inden i reagensposerne.
- Tørremidlet må ikke fjernes fra reagensposerne.
- Luk straks de beskyttende poser med reagenser med lynlåsforseglingen efter hver brug. Fjern eventuel overskydende luft i poserne inden forsegling.
- Brug ikke reagenser, hvis folien er blevet ødelagt eller beskadiget.
- Reagenser fra forskellige poser og/eller sæt og/eller partier må ikke blandes.
- Beskyt reagenser mod fugt. Længerevarende eksponering for fugt kan påvirke produktets ydeevne.
- Hold komponenterne væk fra lys.
- I tilfælde, hvor andre PCR-tests udføres i det samme område i laboratoriet, skal der udvises forsigtighed for at sikre, at VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 ekstraktionssættet eller eventuelle yderligere reagenser, der kræves til testning samt BD MAX™ System, ikke kontamineres. Undgå altid mikrobiel og ribonuklease (RNase)/deoxyribonuklease (DNase) kontaminering af reagenser. Det anbefales at anvende sterile RNase/DNase-fri aerosolresistente engangspipettespidser eller positive fortrængningspipettespidser. Brug en ny spids til hver prøve. Handsker skal udskiftes før håndtering af reagenser og kassetter (BD MAX™ PCR Cartridge).
- For at undgå kontaminering af miljøet med amplikoner må BD MAX™ PCR Cartridge ikke brydes fra hinanden efter brug. Forseglingerne på BD MAX™ PCR Cartridge er designet til at forhindre kontaminering.
- Tilrettelæg en ensrettet arbejdsgang. Den skal begynde i ekstraktionsområdet og derefter flyttes til forstærknings- og detektionsområdet. Prøver, udstyr og reagenser må ikke returneres til det område, hvor det foregående trin blev udført.
- Følg god laboratoriepraksis. Brug beskyttelsestøj, engangshandsker, beskyttelsesbriller og maske. Man må ikke spise, drikke, ryge eller lægge makeup i arbejdsområdet. Vask hænder efter endt test.
- Prøverne skal behandles som potentielt smitsomme og/eller biologisk farlige, samt alle reagenser og materialer, der er blevet eksponeret for prøverne, og skal håndteres i overensstemmelse med de nationale sikkerhedsforskrifter. Træf de nødvendige forholdsregler under indsamling, opbevaring, behandling og bortskaffelse af prøver.

- Prøver og reagenser skal håndteres i et biologisk sikkerhedsskab. Anvend personlige værnemidler (PPE) i overensstemmelse med gældende retningslinjer for håndtering af potentielt smitsomme prøver. Affald bortskaffes i overensstemmelse med lokale retningslinjer.
- Regelmæssig dekontaminering af almindeligt anvendt udstyr anbefales, især mikropipetter og arbejdsflader.
- "I overensstemmelse med Forordning (EF) nr. 1907/2006 (REACH), kræver VIASURE Real Time PCR Detection Kits ikke materialesikkerhedsdatablade (Material Safety Data Sheets) som en del af deres klassificering som værende ufarlige for helbredet og miljøet, fordi de ikke indeholder stoffer og/eller blandinger, som opfylder kriterierne for fareklassificering iht. forordning (EF) nr. 1272/2008 (CLP), eller forefindes i koncentrationer, der er højere end den værdi, der er angivet i den nævnte forordning til deres erklæring.
- Se brugervejledningen til BD MAX™ System for yderligere advarsler, forholdsregler og procedurer.

8. Analysemetode

8.1. Prøveindsamling, opbevaring og transport

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System er blevet testet med nasofaryngeale podninger, som er blevet indsamlet i sterilt Vircell® transportmedium eller i sterilt virustransport- og opbevaringsmedium (Biocomma®) og i universelt transportmedium, alt afhængigt af prøvetypen. Andre typer prøver skal valideres af brugeren.

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

De kliniske prøver skal indsamles, transporteres og opbevares i henhold til relevante laboratorieretningslinjer. For yderligere oplysninger henvises til CDC guideline (CDC-retningslinjer for prøveudtagning. Websted https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf), IDSA-vejledningen (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). En vejledning i anvendelse af mikrobiologilaboratoriet til diagnosticering af smitsomme sygdomme: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94) and García-Lechuz Moya, J.M., González López, J.J., Orta Mira, N., Sánchez Romero, M.I. (2017). Recogida, transporte y procesamiento general de las muestras en el Laboratorio de Microbiología. 2017. 1b. Sánchez Romero, M.I., (coordinadora). Procedimientos en Microbiología Clínica. Cercenado Mansilla, E., Cantón Moreno, R., (editores). *Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC)*.

8.2. Prøveklargøring og DNA-ekstraktion

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

1. Pipettér 400-750 µl nasofaryngeal prøve i et BD MAX™ ExK™ TNA-3 prøvebufferrør, og luk røret med en septumhætte. Der sikres fuldstændig blanding ved at vortex'e prøven ved høj hastighed i 1 minut. Fortsæt til BD MAX™ System Operation betjening.

8.3. PCR-protokol

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

8.3.1. Oprettelse af et PCR-testprogram til VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System

Bemærk: Hvis du allerede har oprettet testen til VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System, kan du springe trin 8.3.1 over og gå direkte til 8.3.2.

1) Vælg fanen "Test Editor" (Testredigering) på skærmen "Run" (Kør) på BD MAX™ System.
2) Klik på knappen "Create" (Opret).
3) På fanen Basic Information (Grundlæggende oplysninger) i vinduet "Test Name" (Navn på test), skal du navngive din test: dvs. VIASURE *Respiratory Virus Mix I.*
4) I rullemenuen "Extraction Type" (Ekstraktionstype), vælg "ExK TNA-3".
5) Vælg "Type 5" i rullemenuen "Master Mix Format".
    a. Bemærk: Produktet kan anvendes i kombination med en ekstra VIASURE til BD MAX test, og vælg derefter "Dual Master Mix Concentrated Lyofized MM with Rehydration Buffer (Type 5)".
6) I "Sample extraction parameters" (Parametre for prøveekstraktion) vælges "User defined" (Brugerdefineret), og prøvevolumen justeres til 950 µl.
7) I "Ct Calculation" (Ct-beregning) vælges "Call Ct at Threshold Crossing" (Beregn Ct når tærsklen krydses).
8) Hvis du kører softwareversion 5.00 eller nyere og har snap-in-rør med stregkodet folie, skal du vælge følgende konfiguration i "Custom Barcodes" (Brugerdefinerede stregkoder):
    a. "Snap-In 2 Barcode" (Snap-In 2-stregkode): 1K (vedrørende *Respiratory Virus Mix I* reaction tube *(*reaktionsrør)).
    b. "Snap-In 3 Barcode" (Snap-In 3-stregkode): 11 (vedrørende Rehydration Buffer tube).
    c. "Snap-In 4 Barcode" (Snap-in 4-stregkode): et andet VIASURE reaction tube (forskellig folie), hvis du vælger formatet "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Afsnit 8.3.1).

9) Indtast følgende parametre på fanen "PCR settings" (PCR-indstillinger): "Channel Settings" (Kanalindstillinger), "Gains" (Stigninger) og "Threshold" (Tærskel) (Tabel 3).
    a. Bemærk: Produktet kan anvendes i kombination med en ekstra VIASURE til BD MAX™ -test, PCR-indstillinger og testtrin skal gennemføres for position 2 (grøn) og position 4 (blå).

Channel (Kanal)	Alias (Alias)	Gain (Gevinst)	Threshold (Tærskel)	Ct Min (Ct Min)	Ct Max (Ct Max)
475/520 (FAM)	SARS-CoV-2	80	150	0	40
530/565 (HEX)	Influenza B	80	150	0	40
585/630 (ROX)	Influenza A	80	150	0	40
630/665 (Cy5)	RSV (A/B)	80	150	0	40
680/715 (Cy5.5)	EIC	80	150	0	35

Tabel 12. "PCR settings" (PCR-indstillinger).

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

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

		False Receiving Channel (Falsk modtagekanal)				
	Channel (Kanal)	475/520	530/565	585/630	630/665	680/715
Excitation Channel (Excitationskanal)	475/520	-	4,0	0,0	0,0	0,0
	530/565	1,0	-	0,0	0,0	0,0
	585/630	0,0	0,0	-	1,0	0,0
	630/665	0,0	0,0	3,0	-	18,0
	680/715	0,0	0,0	0,0	1,5	-

Tabel 13. Parametre for spektral krydstale.

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

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

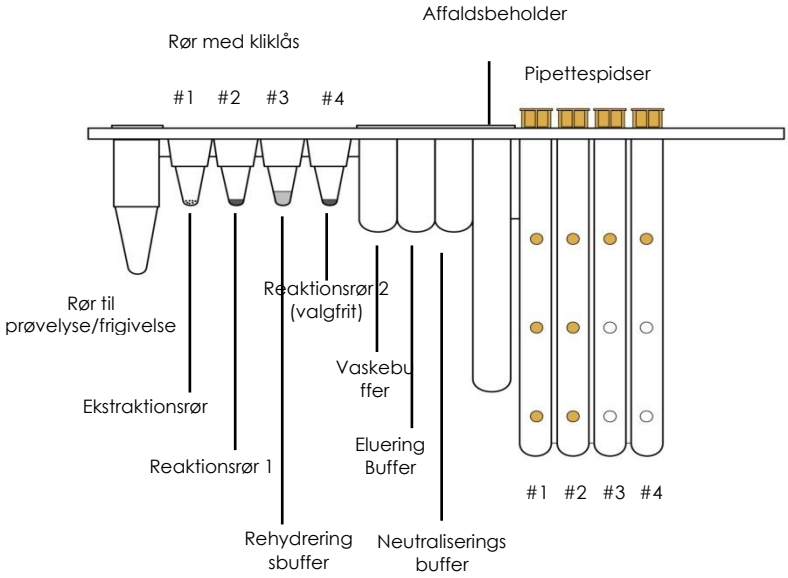
Tabel 14. PCR-protokol.

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

8.3.2. Opsætning af BD MAX™-stativ

1) For hver prøve, der skal testes, fjernes en Unitized Reagent Strips (samlet reagensstrimmel) fra BD MAX™ ExK™ TNA-3 kit. Bank forsigtigt hver strimmel mod en hård overflade for at sikre, at alle væskerne ligger i bunden af rørene, og anbring dem i BD MAX™ Systems prøvestativer.
2) Fjern det nødvendige antal BD MAX™ ExK™ TNA Extraction Tubes (B4) (hvid folie) fra deres beskyttelsespose. Sæt udtræksrøret(-rørene) (hvid folie) i de tilsvarende positioner i TNA-strimlen (fastgør position 1, hvid farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk posen med lynlåsforseglingen.
3) Beregn og adskil det passende antal *Respiratory Virus Mix I* reaction tube (reaktionsrør) (1K folie), og klik dem på plads i deres tilsvarende positioner i strimlen (klik-position 2, grøn farvekodning på stativet. Se Figur 1).
    a. Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    b. Rehydreringen udføres korrekt ved at sørge for, at det frysetørrede produkt ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.
        i. Bemærk: Hvis du vælger formatet "Dual Master Mix Concentrated Lyophilised MM with Rehydration Buffer (Type 5)" (Dobbelt mastermix koncentreret frysetørret MM med rehydreringsbuffer (type 5)) (afsnit 8.3.1), bestemmes og adskilles det passende antal ekstra VIASURE reaktionsrør (forskellig folie) og klikkes fast i deres tilsvarende positioner i strimlen (klik-position 4, blå farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
4) Fjern det nødvendige antal Rehydration Buffer tubes (11 folie), og klik dem fast på deres tilsvarende pladser på strimlen (klik-position 3, ikke-farvet kodning på stativet. Se Figur 1). Fjern overskydende luft, og luk posen med lynlåsforseglingen.
    a. For at sikre, at overførslen udføres korrekt, skal man sørge for, at væsken ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt på hvert rør på en hård overflade for at sikre, at al bufferen er i bunden af røret.

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



8.3.3. BD MAX™ Instrumentopsætning

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

8.3.4. BD MAX™ rapport

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

9. Tolkning af resultater

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

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

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

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

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

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

Resultaterne skal læses og analyseres ved hjælp af følgende tabel:

SARS-CoV-2 (475/520)	Flu B (530/565)	Flu A (585/630)	RSV (A/B) (630/655)	Endogenous Internal Control (680/715)	Fortolkning
+	+	+	+	+/-[1]	**SARS-CoV-2, Flu B, Flu A og RSV (A/B) RNA detekteret[1]**
-	+	+	+	+/-[1]	**Flu B, Flu A, RSV (A/B) RNA detekteret og SARS-CoV-2 RNA ikke detekteret[1]**
+	-	+	+	+/-[1]	**SARS-CoV-2, Flu A, RSV (A/B) RNA detekteret og Flu B RNA ikke detekteret[1]**
+	+	-	+	+/-[1]	**SARS-CoV-2, Flu B, RSV (A/B) RNA detekteret og Flu A RNA ikke detekteret[1]**
+	+	+	-	+/-[1]	**SARS-CoV-2, Flu B, Flu A RNA detekteret og RSV (A/B) RNA ikke detekteret[1]**
+	+	-	-	+/-[1]	**SARS-CoV-2, Flu B RNA detekteret og Flu A, RSV (A/B) RNA ikke detekteret[1]**
+	-	+	-	+/-[1]	**SARS-CoV-2, Flu A RNA detekteret og Flu B, RSV (A/B) RNA ikke detekteret[1]**
+	-	-	+	+/-[1]	**SARS-CoV-2, RSV (A/B) RNA detekteret og Flu B, Flu A RNA ikke detekteret[1]**
-	+	+	-	+/-[1]	**Flu B, Flu A RNA detekteret og SARS-CoV-2, RSV (A/B) RNA ikke detekteret[1]**
-	+	-	+	+/-[1]	**Flu B, RSV (A/B) RNA detekteret og SARS-CoV-2, Flu A RNA ikke detekteret[1]**
-	-	+	+	+/-[1]	**Flu A, RSV (A/B) RNA detekteret og SARS-CoV-2, Flu B RNA ikke detekteret[1]**
+	-	-	-	+/-[1]	**SARS-CoV-2 RNA detekteret[1]**
-	+	-	-	+/-[1]	**Influenza B RNA detekteret[1]**
-	-	+	-	+/-[1]	**Influenza A RNA detekteret[1]**
-	-	-	+	+/-[1]	**RSV RNA detekteret[1]**
-	-	-	-	+[2]	**SARS-CoV-2, Flu B, Flu A og RSV (A/B) RNA ikke detekteret[2]**
-	-	-	-	-[2]	**Resultatet Unresolved (Uløst) (UNR) fremkommer under tilstedeværelse af hæmmere i PCR-reaktionen, eller når der opstår et generelt problem (der ikke rapporteres med en fejlkode) under prøvekørslen og/eller forstærkningstrinnene.[2]**
IND	IND	IND	IND	IND	**Analyseresultatet er Indeterminate (ubestemmeligt) (IND). Skyldes en fejl i BD MAX™ System. Analyseresultat, der vises i tilfælde af en instrumentfejl, der er knyttet til en fejlkode.**
INC	INC	INC	INC	INC	**Analyseresultatet er Incomplete (ufuldstændigt) (INC). Skyldes en fejl i BD MAX™ System. Analyseresultatet vises, hvor en fuldstændig kørsel ikke kunne gennemføres.**

Tabel 15. Prøvefortolkning.
+: Der opstod forstærkning.
-: Der opstod ingen forstærkning.

1 En prøve betragtes som positiv, hvis Ct-værdien er mindre end 40. Den endogene interne kontrol (EIC) viser muligvis eller muligvis ikke et forstærkningssignal. Sommetider er EIC-detektionen ikke nødvendig, fordi et højt kopital for målet kan forårsage præferentiel amplifikation af målspecifikke nukleinsyrer.

2 En prøve anses for negativ, hvis prøven ikke viser noget forstærkningssignal i påvisningssystemet, men den interne kontrol er positiv (Ct ≤ 35). En hæmning af PCR-reaktioner kan udelukkes ved forstærkning af den interne kontrol. I tilfælde af uløste resultater (UNR), manglende internt kontrolsignal i en negativ prøve anbefales det at gentage analysen ved følgende indikationer angivet.

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

BEMÆRK: Nye prøver kan afprøves i samme omgang med gentagne prøver.

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

10. Begrænsninger i testen

- Resultaterne af testen bør vurderes af en læge på baggrund af anamnese, kliniske symptomer og andre diagnostiske tests.
- Selvom denne analyse kan anvendes til andre prøvetyper, er den blevet valideret med nasofaryngeale podninger.
- For at opnå en tilfredsstillende testydeevne skal det frysetørrede produkt ligge i bunden af røret og ikke klæbe til det øverste område af røret eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.
- Et udseende af reaktionsblandingen i stabiliseret format, som normalt findes i bunden af røret, forskelligt fra det sædvanlige (uden konisk form, inhomogent, mindre/større i størrelse og/eller farve forskellig fra hvidlig) ændrer ikke testens funktionalitet.
- Testens kvalitet afhænger af prøvens kvalitet; korrekt ekstraheret nukleinsyre fra luftvejsprøver skal ekstraheres.
- Denne test er en kvalitativ test og giver ikke kvantitative værdier eller angiver antallet af tilstedeværende organismer.
- Meget lave målniveauer under detektionsgrænsen kan påvises, men resultaterne er muligvis ikke reproducerbare.
- Der kan opnås falske positive resultater på grund af krydskontaminering med prøver med muligt indhold af SARS-CoV-2, Influenza B, Influenza A eller RSV (A/B*)* og høje koncentrationer af mål-RNA eller med prøver, som er kontaminerede som følge af PCR-produkter fra foregående reaktioner.
- De specifikke kombinationer af primere og prober til detektion af generne *N* og *ORF1ab* hos SARS-CoV-2, genet *M1* hos Influenza B, genet *M1* hos Influenza A og genet *N* hos RSV (type A og B) der anvendes i VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™, har ingen betydende homologi med det human genom, den humane mikroflora eller andre mikroorganismer i luftvejene, når de kombineres. Det ville resultere i, at der kunne fremkomme falske positive.
- Falsk-negative resultater kan skyldes flere faktorer og kombinationer heraf, herunder:

- Forkerte metoder til indsamling, transport, opbevaring og/eller håndtering af prøver.
- Forkerte behandlingsprocedurer (herunder RNA-ekstraktion).
- Nedbrydning af RNA under forsendelse/opbevaring og/eller behandling af prøver.
- Mutationer eller polymorfisme i de primer- eller probe-bindende regioner kan påvirke detektionen af nye eller ukendte typer af SARS-CoV-2, Influenza B, Influenza A or RSV (A/B).
- Antal virus, som er under analysens detektionsgrænse.
- Tilstedeværelsen af qPCR-hæmmere eller andre typer interfererende stoffer. Virkningerne af vacciner, antivirale midler, antibiotika, kemoterapeutika eller immunsuppressive lægemidler, der bruges til at forhindre COVID-19, gripe og RSV, eller som anvendes under behandlingen af infektionen, er ikke blevet evalueret.
- Manglende overholdelse af brugsanvisningen og analyseproceduren.

- Nogle prøver viser eventuelt ikke *RNase P*-amplifikationskurver på grund af lave humane celletal i den oprindelige kliniske prøve. Et negativt IC-signal udelukker ikke tilstedeværelsen af SARS-CoV-2, Influenza B, Influenza A eller RSV (A/B) i en klinisk prøve.
- Et positivt testresultat indikerer ikke nødvendigvis tilstedeværelse af levende virus, og det betyder ikke, at disse virus er infektiøse, eller at de er den underliggende årsag til kliniske symptomer. Imidlertid er et positivt resultat indikativt for tilstedeværelse af virale målsekvenser.
- Hvis diagnostiske tests for andre luftvejssygdomme er negative, og patientens kliniske fremtoning og de epidemiologiske oplysninger antyder en mulig infektion med SARS-CoV-2, Influenza B, Influenza A eller RSV (A/B), skal et falsk negativt resultat overvejes og gentestning af patienten tages op til revision.
- Et negativt resultat udelukker ikke nødvendigvis tilstedeværelsen af*mål-RNA* i en klinisk prøve. Hvis kliniske observationer, patienthistorik og epidemiologiske oplysninger antyder, at der er infektion med SARS-CoV-2, Influenza B, Influenza A eller RSV (A/B), bør gentestning med et større prøvevolumen overvejes.
- Gentestning vil være nødvendig, hvis der opnås uløste, ubestemmelige eller ufuldstændige resultater under brug af VIASURE *Respiratory Virus Mix I Real* Time PCR Detection Kit for BD MAX™ System. Uløste resultater kan skyldes tilstedeværelsen af hæmmere i prøven eller forkert rehydrering af frysetørrede reaktionsblandingsrør. Hvis der opstår en instrumentfejl, kan det medføre ubestemmelige eller ufuldstændige resultater.

11. Kvalitetskontrol

VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System indeholder en endogen intern kontrol (EIC) i hvert reaktionsrør, som bekræfter korrekt præstation af teknikken.

12. Ydelseskarakteristika

12.1. Klinisk sensitivitet og specificitet

Den kliniske præstation af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System blev testet ved brug af kliniske prøver (nasofaryngeale podninger) fra patienter med klinisk mistanke om infektion med SARS-CoV-2, Flu A/B og/eller RSV A/B. Resultaterne var følgende:

	Center	Prøvetype	Arbejdsgang	Mål
1	CerTest Biotec S.L. in collaboration with the Biobank of the Sistema de Salud de Aragón (BSSA) and the Microbiology and Parasitology Department of Hospital Universitario Central de Asturias	Nasofaryngeale podninger	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	SARS-CoV-2
				Influenza B
				Influenza A
				RSV (A/B)

Tabel 16. Sted, prøvetype, arbejdsgang og mål.

Sande positive og negative værdier, falske positive og negative værdier, sensitivitet og specificitet af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System blev beregnet i forhold til hver sammenligningsanalyse, som vist i følgende tabel:

Center	Komparatoranalyse	Mål	TP	TN	FP	FN	Følsomhed	Specificitet
1	TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific) eller VIASURE *SARS-CoV-2* Real time PCR Detection Kit, + efterfølgende sekventering af hele genomet.	SARS-CoV-2	127	625	1	3	0,977 (0,934-0,995)	0,998 (0,991-1)
	Cobas® Influenza A/B & RSV nukleinsyretest til brug på cobas® Liat® System (cobas® Influenza A/B & RSV)	Influenza B	18	738	0	0	1 (0,815-1)	1 (0,995-1)
		Influenza A	49	704	1	2	0,961 (0,865 – 0,995)	0,999 (0,992-1)
		RSV (A/B)	50	706	0	0	1 (0,929-1)	1 (0,995-1)

Tabel 17. Sande positive (TP) og negative (TN) værdier, falske positive (FP) og negative (FN) værdier, sensitivitet og specificitet af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System.

Resultatet bekræfter, at SARS-CoV-2, Influenza B, Influenza A og RSV (A/B) kan detekteres ved brug af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytisk sensitivitet

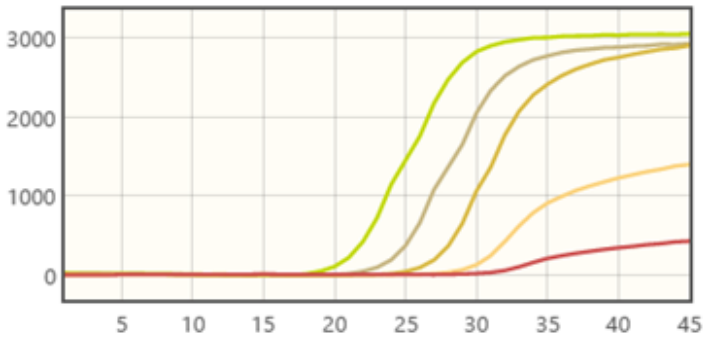
Resultater for detektionsgrænsen (LoD) for nasofaryngeale prøver med VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System med en positiv detektionsgrad på ≥ 95 % er som følger:

a) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse (LoD) på 5,01 IE (internationale enheder)/µl for SARS-CoV-2.
b) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse (LoD) på 1,8 x 10^2 $CEID_{50}$ (median infektiøs dosis i kyllingeembryo)/ml for Influenza B.
c) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse (LoD) på $10^{-0.5}$ $TCID_{50}$ (median infektiøs dosis i vævskultur) /ml for Influenza A.
d) VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse (LoD) på 4 genomkopier/µl for RSVA og RSVB.

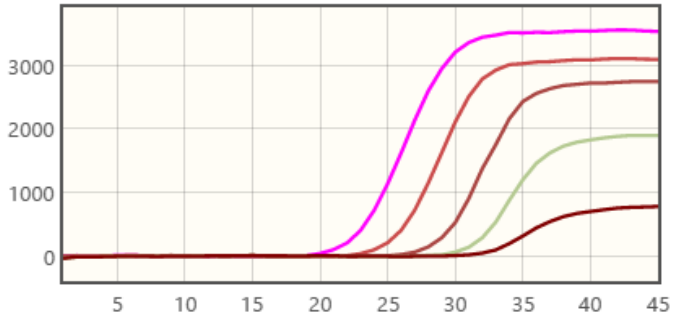
Bemærk: Detektionsgrænsen blev beregnet med et prøvevolumen på 400 µl.

Eksempler på amplifikationskurver som resultat af kørsel af en analyse på BD MAX™ System er vist herunder.

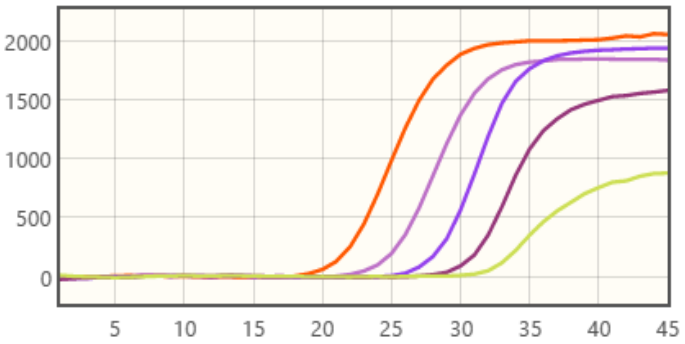
Figur 2. Fortyndningsserie af SARS-CoV-2-templates ($5x10^5$-$5x10^0$ kopier pr. reaktion) kørt på BD MAX™ System (475/520 (FAM) kanal).



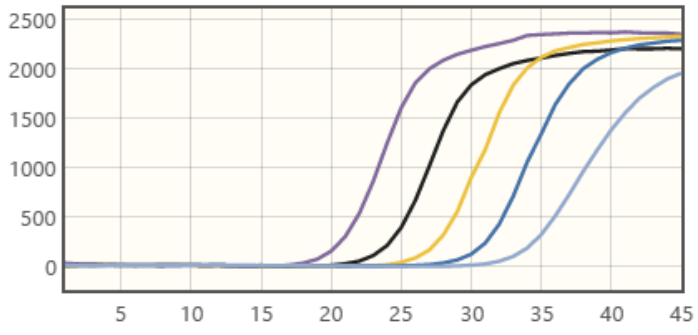
Figur 3. Fortyndningsserie af Influenza B-templates ($5x10^5$-$5x10^0$ kopier pr. reaktion) kørt på BD MAX™ System (530/565 (HEX) kanal).



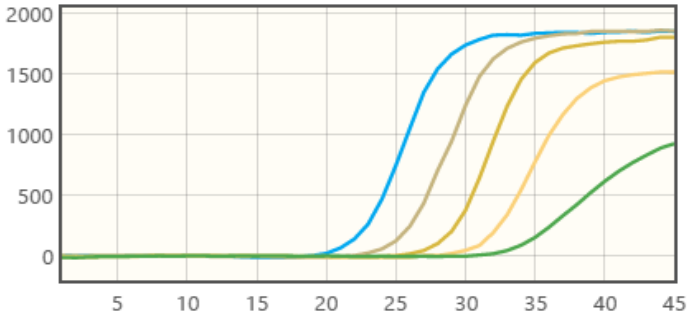
Figur 4. Fortyndningsserie af Influenza A-templates ($5x10^5$-$5x10^0$ kopier pr. reaktion) kørt på BD MAX™ System (585/630 (ROX) kanal).



Figur 5. Fortyndningsserie af RSVA-templates ($5x10^5$-$5x10^0$ genomkopier pr. reaktion) kørt på BD MAX™ System (630/665 (CY5) kanal).



Figur 6. Fortyndningsserie af RSVB-templates ($5x10^5$-$5x10^0$ genomkopier pr. reaktion) kørt på BD MAX™ System (630/665 (CY5) kanal).



12.3. Analytisk specificitet

Specificiteten af *Respiratory Virus Mix I*-analysen blev bekræftet ved at teste et panel bestående af forskellige mikroorganismer, der er forbundet med luftvejsinfektioner. Der blev ikke påvist krydsreaktivitet mellem nogen af følgende testede mikroorganismer:

Krydsreaktivitetstest					
Human Adenovirus-type 1-5, 8, 15, 31, 40 og 41	-	Enterovirus Coxsackievirus A24, A9 og B3	-	Mycoplasma pneumoniae	-
Bocavirus	-	Enterovirus Echovirus 30	-	Mycobacterium tuberculosis	-
Bordetella bronchiseptica	-	Enterovirus 68 , 71	-	Human parainfluenza 1, 2, 3 og 4 vira	-
Bordetella holmesii	-	Haemophilus influenzae MinnA	-	Pneumocytis jirovecii type A1 og g885652	-
Bordetella parapertussis	-	Legionella bozemanii	-	Human rhinovirus	-
Bordetella pertussis	-	Legionella dumoffii	-	SARS Coronavirus-stamme Frankfurt 1	-
Chlamydia caviae	-	Legionella longbeachae	-	Staphylococcus aureus	-
Chlamydia psittaci genotype A og C	-	Legionella micdadei	-	Staphylococcus epidermidis	-
Chlamydophila pneumoniae CM-1	-	Legionella pneumophila	-	Streptococcus pneumoniae	-
Human coronavirus 29E OC43, NL63 og HKU1	-	Human metapneumovirus A og B	-	Streptococcus pyogenes	-
MERS Coronavirus	-	Moraxella catarrhalis	-	Streptococcus salivarius	-

Tabel 18. Referencepatogene mikroorganismer, der blev brugt i denne undersøgelse.

12.4. Analytisk reaktivitet

Reaktiviteten af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **SARS-CoV-2** blev evalueret med RNA ekstraheret fra human 2019-nCoV-stamme BetaCoV/Germany/BavPat1/2020 p.1, human 2019-nCoV-stamme 2019-nCoV/Italy-INMI1, syntetiske RNA-kontroller for MT007544.1-variant (SARSCoV2 isolate Australia/VIC01/2020), MN908947.3 variant (SARS-CoV-2 isolate Wuhan-Hu-1), alfa-variant (B.1.1.7 England/MILK-9E05B3/2020), beta-variant (B.1.351 South Africa/KRISP-EC-K005299/2020), gammavariant (P.1 Japan (Brazil) /IC-0564/2021) og kappavariant (B.1.617.1 India/CT-ILSGS00361/2021), og varmeinaktiveret SARSCoV-2-stamme 2019nCoV/USAWA1/2020 (ATCC® VR1986HK™), og bestrålet cellelysat fra 2019-nCoV/USA-WA1/2020, og

lyofiliserede cellelysater fra BetaCoV/Berlin/ChVir1670/2020_IsolatBER, BetaCoV/Munich/ChVir984/2020 and BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020_IsolatBER, og viste positive resultater.

Reaktiviteten af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **Influenza B** blev evalueret med RNA ekstraheret fra følgende stammer: B/Phuket/3073/2013 virus, B/Brisbane/60/2008 virus, Influenza B/Florida/04/06 virus, B/Pennsylvania/7/2007 (Yamagata-slægt), B/Santiago/4364/2007 (Yamagata-slægt) virus, B/Brisbane/3/2007 (Yamagata-slægt) virus, B/Pennsylvania/5/2007 (Victoria-slægt), B/Victoria/304/2006 (Victoria-slægt) virus, B/Bangladesh/3333/2007 (Yamagata-slægt) virus, og viste positive resultater.

Reaktiviteten af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **Influenza A** blev evalueret med RNA ekstraheret fra følgende stammer: A/Switzerland/9715293/2013 (H3N2) virus, A/Thüringen/5/2017 (H3N2) virus, A/DE-SH/Reiherente/AR8444/ 2016(H5N8) virus, A/Anhui/1/2013 (H7N9) virus, A/Michigan/45/2015 (H1N1 pdm09) virus, A/California/7/2009 (H1N1) virus, A/California/7/2009 (H1N1pdm09) virus, A/South Australia/55/2014 virus, Switzerland/9715293/2013 (H3N2) IVR-175 virus, A/Singapore/GP1908/2015 IVR-180 virus, A/Hong Kong/4801/2014 NYMC X-263B virus, Influenza A/New Caledonia/20/99 (H1N1) virus, A/Brisbane/59/2007 (H1N1) virus, A/South Dakota/6/2007 (H1N1) virus, A/Hawaii/31/2007 (H1N1) virus, A/Qatar/1123/2007 (H1N1) virus, A/Cambodia/0371/2007 (H1N1) virus , Influenza A Virus, A/Brisbane/10/2007 (H3N2) virus, Influenza A Virus, A/Taiwan/760/2007 (H3N2) virus, Influenza A Virus, A/Texas/71/2007 (H3N2) virus, A/Brisbane/10/2007 (H3N2) IVR-147 virus, A/Brisbane/59/2007 (H1N1) IVR-148 virus, A/South Dakota/6/2007 (H1N1) X-173 virus, A/California/07/2009 (H1N1)pdm09 virus, A/California/08/2009 (H1N1)pdm09 virus, A/New York/18/2009 (H1N1)pdm09 virus, A/Mexico/4108/2009 (H1N1)pdm09 virus, A/California/07/2009 (H1N1 pdm09) NYMC X-179A virus, A/Victoria/2570/2019 IVR-215 virus og A/Cambodia/e0826360/2020 IVR-224 virus og viste positive resultater.

Reaktiviteten af VIASURE *Respiratory Virus Mix I* Real Time PCR Detection Kit for BD MAX™ System for **RSV** blev evalueret med RNA ekstraheret fra respiratorisk syncytial virus A (stamme A-2) og respiratorisk syncytial virus B (stamme 9320), og viste positive resultater.

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

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

Trademarks

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Change Control / Ændringskontrol		
Version No. / Versionsnr.	**Changes / Ændringer**	**Date / Dato**
00	Original version / Original version.	25/07/2022
01	"Spectral Cross Talk" values of table 4 have been updated / "Spectral Cross Talk"-værdierne i tabel 4 er blevet opdateret	02/08/2022

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

Revision: 02nd August 2022.

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