VIASURE by certest

Real Time PCR Detection Kit

Respiratory Virus Extended Mix for BD MAX™ System

Instructions for use

CE IVD
2797

These instructions for use apply to the following references:

PRODUCT	REFERENCE
VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System	444221

Table A 1. Reference for product to be used with the BD MAX™ System.

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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.

Content

ENGLISH

1. Intended purpose

The VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System is an automated RT-qPCR test designed for the simultaneous qualitative detection of RNA/DNA from SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and adenovirus in 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 diagnosis of infection with the above-mentioned microorganisms in combination with patient's clinical signs and symptoms and/or epidemiological risk factors. Positive results are indicative of the nucleic acids (NA) target's presence but do not preclude the presence of other pathogens not detected by the test. Negative results do not preclude the presence of the NA targets and should not be used as the sole basis for treatment, or other patient management decisions. The assay uses the BD MAX™ System for automated extraction of RNA/DNA and subsequent RT-qPCR employing the reagents provided combined with universal reagents and disposables for the BD MAX™ System. RNA/DNA is extracted from samples. Complementary DNA (cDNA) is synthetized, and DNA/cDNA are amplified using RT-qPCR and detected using specific primers and fluorescent reporter dye probes for SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and adenovirus.

The product is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures (including training on the Real Time PCR instrument (thermocycler) and nucleic acid extraction system).

2. Summary and Explanation

The Severe Acute Respiratory Syndrome Coronavirus 2, or SARS-CoV-2 as commonly known, is the respiratory virus surged in the end of 2019 responsible of COVID-19 disease, which was later characterized as a global pandemic by the WHO (Fernández-Pérez et al., 2021; Hu et al., 2021; WHO | World Health Organization, 2023a). This novel coronavirus is a single-stranded RNA virus that has been included into the *Coronaviridae* family, beta genus (Fernández-Pérez et al., 2021). SARS-CoV-2 infection can affect both adults and children, although people over 60 years of age and those who present pre-existing medical conditions are more prone to suffer a more serious form of COVID-19 (WHO | World Health Organization, 2023a). Infection may be asymptomatic, or give rise to several symptoms from low to high rate of intensity and severity, very similar to influenza illness (CDC |, 2023; Safiabadi Tali et al., 2021).

Influenza is the acute respiratory infection caused by the influenza virus, which affects the respiratory tract, and can be found in circulation worldwide (Uyeki et al., 2022; WHO | World Health Organization, 2023b). Seasonal influenza, in particular, is the one triggered by seasonal Influenza A and B viruses, both responsible for the seasonal epidemics that normally occur in winter periods in temperate climates, and throughout the year in tropical areas (Tyrrell et al., 2021; Uyeki et al., 2022; WHO | World Health Organization, 2023b). Influenza viruses, part of the *Orthomyxoviridae* family, are eight-segmented negative-sense single-strand RNA viruses that encodes 12 viral proteins (Krammer et al., 2018; Uyeki et al., 2022). The viral envelope, derived from the host plasma membrane, consists of a lipid bilayer containing the transmembrane proteins haemagglutinin (HA), neuraminidase (NA), viral nucleoprotein (NP), matrix protein (M1) and membrane protein (M2) (Krammer et al., 2018).

The Respiratory Syncytial Virus (RSV) is another respiratory and seasonal virus that affects the lower respiratory tract in all age groups (WHO | World Health Organization, n.d.). This single-stranded non-segmented negative-sense RNA virus, member of the *Pneumoviridae* family, is known for affecting mainly toddlers under 2 years old, but also may affect severely to adults older than 65 years and/or immunocompromised people or with specific comorbidities (Abu-Raya et al., 2023; Bergeron & Tripp, 2021; WHO | World Health Organization, n.d.).

The diagnosis of mentioned respiratory diseases is challenging because they usually share common symptoms (Uyeki et al., 2022). Thus, accurate diagnosis is key, not only to know the cause of the disease, but also to anticipate epidemic/pandemic waves and to mitigate the collateral impact on healthcare and economic systems (Safiabadi Tali et al., 2021; Uyeki et al., 2022). Many types of diagnostic tests are available (Point-of-care or rapid antigen detection); however, RT-PCR is more sensitive and specific, and allows the combine detection of many co-circulating respiratory viruses at the same time, reducing the time of diagnosis (Uyeki et al., 2022).

Lower respiratory tract disease accounts for, approximately, four million deaths annually worldwide. A wide variety of viruses can be held responsible for this, one of them being Coronaviruses, which belong to the *Coronaviridae* family (Friedman et al., 2018). These globally distributed viruses are large, enveloped viruses containing a single-stranded RNA genome of positive polarity (Lim et al., 2016; Zeng et al., 2018). They are directly related to diseases for the respiratory tract, gastrointestinal tract and Central Nervous System. Coronaviruses are characterized for their division into three serotypes or groups. Groups 1 and 2 refer to mammalian Coronaviruses, whilst group 3 consists of avian Coronaviruses. Common human Coronaviruses are the HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 strains (Zeng et al., 2018). HCoVs are difficult to detect via common diagnosing methods because they are normally co-detected with other respiratory viruses, such as HRSV or influenzas (Gaunt et al., 2010). Given all this, Real Time PCR is one of the preferred methods to diagnose Coronaviruses due to its specificity. More concretely, Real Time PCR that sets the *N* gene for strains 229E, OC43 and NL63, plus the *rep* gene for strain HKU1 as targets.

Parainfluenza viruses (PIV or HPIV in humans) are part of the Paramyxoviridae family and are classified genetically and antigenically into four types. These viruses can lead to respiratory infections in infants, children, and adults, with the type of infection and specific symptoms varying by type. HPIV-1 and HPIV-2 both cause upper and lower respiratory illnesses, such as colds and croup, with HPIV-1 most commonly found in children. HPIV-3 is more frequently linked to lower respiratory conditions like bronchiolitis, bronchitis, and pneumonia. HPIV-4 is less commonly recognized but can still cause mild to severe respiratory illnesses (Henrickson, 2003). Parainfluenzas are medium size enveloped virus, and their genomes are organized on a single negative-sense strand of RNA that encodes at least six common structural proteins. These viruses carry two envelope glycoproteins: HN, containing both haemagglutinin and neuraminidase activity, and F, carrying fusion activity (Henrickson, 2003).

Viral culture in combination with immunofluorescence is the traditional method for diagnosis but it is time consuming (Templeton et al., 2004). Antigen detection tests are widely used but they are less sensitive and specific than other diagnostics tools such as Real-time PCR assays (Jansen et al., 2011; Templeton et al., 2004), which currently are being considered one of the best methods of choice.

Adenoviruses belong to the *Adenoviridae* family of non-enveloped and double-stranded (dsDNA) viruses (Datta, 2023; Ison & Hayden, 2016). There are more than 50 immunologically distinct human Adenovirus serotypes (Lynch & Kajon, 2016) classified into 7 species (Adenovirus-A to Adenovirus-G) that can cause human infections ranging from respiratory diseases (Adenovirus-E, C and some B species) to digestive tract infections (mainly species Adenovirus-A and F), urinary tract infections (other Adenovirus-B species) and conjunctivitis (Adenovirus-D) (Buckwalter et al., 2012; Datta, 2023). Transmission can occur via inhalation of aerosolized droplets, direct conjunctival inoculation, fecal-oral spread, or exposure to infected tissue or blood(Ison & Hayden, 2016).

Human Metapneumoviruses belong to the *Paramyxoviridae* family (Schuster & Williams, 2013) and are an important cause of upper and lower respiratory infection. Metapneumovirus is an enveloped, single-stranded, negative-sense RNA virus. Clinical symptoms of Metapneumovirus include cough, fever, nasal congestion, and shortness of breath, and may progress to bronchiolitis or pneumonia (Uddin & Thomas, 2020). Metapneumovirus is mainly transmitted by infectious airborne droplets, and it has been reported as the second most frequently found virus in respiratory infections, with children younger than five years being the most susceptible to infection (Schuster & Williams, 2013).

Diagnosis can be problematic, as a wide range of pathogens can cause acute respiratory infections presenting with similar clinical syndromes. They were initially identified by cell culture, but diagnosis through this procedure is time-consuming until the development of the cytopathic effect. Serological tests may be useful in epidemiological investigations but are of limited practical value in individual patients (Datta, 2023; Ison & Hayden, 2016; Lynch & Kajon, 2016; Schuster & Williams, 2013). Therefore, real-time (RT)-PCR is

currently the method used for Adenovirus and Metapneumovirus identification, due to its high sensitivity and specificity.

3. Principle of the procedure

VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System is designed for the simultaneous qualitative detection of nucleic acid from SARS-CoV-2, Influenza B, Influenza A, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and adenovirus in nasopharyngeal swabs. The detection is a one-step RT-qPCR format where the reverse transcription and the subsequent amplification of the specific targeted sequence occur in the same reaction well. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase. After the cDNA is synthetized or the DNA is isolated, the identification of these microorganisms is performed by the amplification of a conserved region of *N* and *ORF1ab* genes of SARS-CoV-2, *M* gene (matrix protein 1 (M1)) for Influenza A/B, the *HA* gene for Influenza A H1N1 subtype, *N* gene of RSV (types A and B), *HN* gen of parainfluenza (types 1, 2 and 3), *F* gene of parainfluenza (type 4), *N* gen of coronavirus (229E, NL63, HKU1 and OC43), *F* gen of metapneumovirus and *hexon* gen of adenovirus, using specific primers and fluorescent-labelled probes.

VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System is based on the 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 target template. This fluorescence is measured on the BD MAX™ System.

VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System contains in each tube all the necessary components for real-time PCR assay (specific primers/probes, dNTPs, buffer, polymerase and retrotranscriptase) in a stabilized[1] format, as well as an **endogenous internal control (EIC)** (human *RNAse P* gene) to follow-up the integrity of the sample, to monitor the extraction process and/or discard the inhibition of the polymerase activity. 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.

[1] Please note that both "stabilized", and "lyophilized" terms are used indistinguishable and as synonyms throughout the entire document.

Target		Channel	Gene
Respiratory Virus Mix I	SARS-CoV-2	475/520	*N* and *ORF1ab* gene
	Influenza B	530/565	*M1* gene
	Influenza A	585/630	*M1* and *HA* genes
	RSV (A/B)	630/665	*N* gene
	Endogenous Internal Control (EIC)	680/715	Human *RNase P* gene
Respiratory Virus Mix II	Parainfluenza (types 1, 2 and 3)	475/520	*HN* gene
	Parainfluenza (type 4)		*F* gene
	Coronavirus (229E, NL63, HKU1 and OC43)	530/565	*N* gene
	Metapneumovirus	585/630	*F* gene
	Adenovirus	630/665	*Hexon* gene
	Endogenous Internal Control (EIC)	680/715	Human *RNase P* gene

Table 1. Target, channel and genes.

4. Reagents provided

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

Reagent/Material	Description	Concentration Range	Barcode	Amount
Respiratory Virus Mix I reaction tube	Lyoprotectors and Stabilizers	±6 g/100 mL*	1K foil	2 pouches of 12 transparent tubes
	Nucleotide triphosphate (dNTPs)	±1 mM*		
	Primers and Probes	0.2-1 nMol/µL*		
	Enzymes	10-100 U/rxn*		
Respiratory Virus Mix II reaction tube	Lyoprotectors and Stabilizers	±6 g/100 mL*	1M foil	2 pouches of 12 transparent tubes
	Nucleotide triphosphate (dNTPs)	±1 mM*		
	Primers and Probes	0.2-1 nMol/µL*		
	Enzymes	10-100 U/rxn*		
Rehydration Buffer tube	Saline Solution Mixture	±13 mM	11 foil	1 pouch of 24 transparent tubes
	Buffer (TRIS, pH)	±67 mM		

Table 2. Reagents and materials provided in VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System with Cat. Nº. 444221.
* For component in stabilized format, the concentration range means after rehydration.

5. Reagents and equipment to be supplied by the user

The following list includes the materials that are required for use but not included in the VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System.

- Real-time PCR instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442827 or 442828).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).

- Nuclease-free water.
- Filter tips.
- Powder-free disposable gloves.

Optional:

- External control materials can be run as part of the quality control procedure of the assay performance. Commercially available control material and/or samples previously characterized as positive or negative can be used as external positive control (EPC) or external negative control (ENC), respectively. The selection and validation of the EPC and ENC must be done according to applicable local, state, and/or federal regulations and the laboratory's standard Quality Control procedures. Additionally, when using commercially available control material the user must follow the respective instructions for use.

VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System has been validated using ExK™ TNA-3 (Ref: 442827 or 442828) on the real-time PCR instrument BD MAX™ System.

Samples selected for the evaluation of the device were nasopharyngeal (NF) specimens collected using a flexible nylon sterile swab (hereafter referred to as nasopharyngeal swab). After that, the swab is placed into the tube of BD™ Universal Viral Transport System (UVT, SKU: 220220) or Universal Transport Media® (UTM®) (Copan).

6. Transport, storage and use conditions

- The kits can be shipped and stored at 2-30°C until the expiration date which is stated on the kit label.
- Avoid vibrations during transport to prevent liquid leakage.
- After opening the aluminum pouches which contain the reaction tubes, the product can be used up to 28 days at 2-30°C. Keep the vial away from light.

The following table summarises the conditions for transport, storage and use of the overall kit and each component:

Component	Transport Conditions	Storage Conditions	In-use conditions
Entire VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System	2-30°C during the shelf life stated in the kit label.	**Before use:** 2-30°C during the shelf life stated in the kit label.	** See in-use conditions of each component.*
Respiratory Virus Mix I reaction tube (1K foil)		**Before use:** 2-30°C during the shelf life stated in the kit label. **Once pouch is opened with the silica gel**: 2-30°C for up to 28 days.	Room temperature.
Respiratory Virus Mix II reaction tube (1M foil)		**Before use:** 2-30°C during the shelf life stated in the kit label. **Once pouch is opened with the silica gel**: 2-30°C for up to 28 days.	Room temperature.
Rehydration Buffer tube		**Before use:** 2-30°C during the shelf life stated in the kit label. **Once pouch is opened with the silica gel**: 2-30°C for up to 28 days.	Room temperature.

Table 3. Summary of the conditions for transport, storage and use of the VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System and each component.

7. Precautions for users

- The product is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- For *in vitro* diagnostic use.
- Instructions for use of the VIASURE product and the BD MAX™ System User's Manual must be read carefully before using the VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. Do not perform the assay until the information about procedures, safety precautions and limitations described therein have been understood.
- 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.
- 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.
- Close protective pouches of reagents promptly with the zip seal after each use to protect the master mix from sunlight. Remove any excess air in the pouches prior to sealing.
- Protect reagents against humidity. Prolonged exposure to humidity may affect product performance.
- To avoid label deterioration, do not use the product near solvents.
- 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.
- Make sure reaction tube and rehydration buffer tube are snapped into place securely during the BD MAX™ rack set up.
- 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 Extended Mix* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, or 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 for BD MAX™ System 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. A statement declaring no requirement of Material Safety Data Sheet could be requested to Certest Biotec S.L.
- Make sure that the definition of the PCR test program on the BD MAX™ System is done following the instructions in the section 'PCR protocol' (Sample extraction parameters, custom barcodes, PCR settings, etc.).
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.
- The certificate of analysis is not included with the device; however, it could be downloaded from Certest Biotec S.L. website (www.certest.es) in case of need.

8. Test procedure

8.1. Sample collection, transport and storage

The VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System has been tested in nasopharyngeal swabs collected with sterile flexible nylon placed immediately into a sterile tube

containing 3 ml of BD™ Universal Viral Transport System (UVT, SKU: 220220)[2] or Universal Transport Media® (UTM®) (Copan)[3]. 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, clinical samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type). After collection, specimens should be placed in a biohazard bag and should be transported and processed as soon as possible to guarantee the quality of the test. The specimens should be transported at Room Temperature (RT) for up to 2 hours, or at 4°C for up to 5 days, following the local and national regulations for the transport of pathogen material. For long term transport (more than 5 days), we recommend shipping at -20°C or lower[4]. Specimens submitted for molecular testing must be stored in controlled conditions so that nucleic acids do not degrade during storage. It is recommended to use fresh specimens for the test, but in the event that this is not possible or in the case of a retrospective study, the samples should be stored preferentially at -70 or -80 °C and, as a second option, at -20 °C[5]. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

The clinical specimens must be collected, transported and stored according to appropriate laboratory guidelines and/or laboratory policy manuals. As examples, refer to 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) or Sánchez-Romero, M. I., García-Lechuz Moya, J. M., González López, J. J., & Orta Mira, N. (2019). Recogida, transporte y procesamiento general de las muestras en el laboratorio de Microbiología. Enfermedades Infecciosas y Microbiología Clínica, 37(2), 127–134. https://doi.org/10.1016/j.eimc.2017.12.002.

Please note: Specimen collection, transport, and storage conditions indicated above are suggested based on the recommendations for nasopharyngeal samples intended to be used for nucleic acid detection, as appear in the referenced SEIMC recommendations report for general collection and transport procedures in clinical microbiology and the IDSA authoritative guide. Nevertheless, we recommend following laboratory guidelines, and/or laboratory policy manual for proper transport and preservation of samples.

An internal specimen stability study was conducted with VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System using negative nasopharyngeal swab collected in BD™ Universal

[2] BD universal viral transport system. https://www.bd.com/en-us/products-and-solutions/products/product-families/bd-universal-viral-transport-system

[3] https://www.copangroup.com/product-ranges/utm/

[4] 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))

[5] Sánchez-Romero, M. I., García-Lechuz Moya, J. M., González López, J. J. & Orta Mira, N. Collection, transport and general processing of clinical specimens in Microbiology laboratory. Enfermedades Infecc. y Microbiol. Clin. (English ed.) 37, 127–134 (2019).

Viral Transport System positive for the product targets, each strain at a concentration of 2-3xLoD. The stability was analysed by means of three different assays: primary stability (25ºC: 24 and 48 hours; 4ºC: 1, 2 and 7 days; -20ºC: 2, 3 and 6 months), stability in the sample buffer tube (3 and 7 days at 25° and 4ºC) and nested stability (samples were incubated at 4ºC and 25ºC for 48 hours, and after that, these samples were added to the SBT and analysed after 3 and 7 days at 4ºC and 25ºC). Besides samples were analysed after going through five freezing (at -20ºC) and thawing (at 25ºC) cycles for one week. Results showed a good performance of samples stored at all conditions tested.

8.2. Sample preparation and NA extraction

Perform the sample preparation according to the recommendations in the instructions for use of the extraction kit used, BD MAX™ ExK™ TNA-3.

1. Pipette 400 µL of sample 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. Ensure that vortexing is performed a few minutes before starting the run. Proceed to BD MAX™ System Operation.

Note that application-specific extraction preparation procedures should be developed and validated by the user and that some other samples may require pre-processing.

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 Extended Mix* Real Time PCR Detection Kit for BD MAX™ System

Note: If you have already created the test for the VIASURE *Respiratory Virus Extended Mix* 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.

In the "Basic Information" tab:

3) Within the "Test Name" field, name your test: i.e. VIASURE Resp Virus.

   Note: The test name must be unique and must have a maximum of twenty characters.

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)". When Dual Master Mix is selected, the tab configuration on the

right of the “Test Editor” tab changes. There are extra tabs for “PCR settings”, “Melt settings” and "Test Steps" considering both snap-in tubes.

6) In the “Sample Extraction Parameters” field select “User Defined” and adjust the following parameters values (table 4).

Sample Extraction Parameters	Value (units)
Lysis Heat Time	10 min
Lysis Temperature	60°C
Sample Tip Height	1600 steps
Sample Volume	950 µL
Wash Volume	500 µL
Neutralization Volume	N/A
DNase Heat Time	N/A

Table 4. Parameters of sample extraction performed with BD MAX™ ExK™ TNA-3.

7) In the “Ct Calculation” field select “Call Ct at Threshold Crossing” (selected by default).

8) If running software version 5.00 or higher and have barcoded foil snap-in tubes, in the “Custom Barcodes” field 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: 1M (concerning *Respiratory Virus Mix II* reaction tube).

In the “PCR Settings” tabs:

9) In “PCR Settings” field enter the following parameters described in Tables 5 and 6 for snap-in 2 (green color coding on the rack) and snap-in 4 (blue color coding on the rack), respectively: “Alias” (up to seven alphanumeric characters), “PCR Gain”, “Threshold”, “Ct Min” and “Ct Max”.

Channel	Alias	PCR Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	SARS	80	150	0	40
530/565 (HEX)	FLUB	40	150	0	40
585/630 (ROX)	FLUA	80	150	0	40
630/665 (Cy5)	RSV	80	150	0	40
680/715 (Cy5.5)	EIC	80	150	0	35

Table 5. PCR settings for snap-in 2.

Channel	Alias	PCR Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	HPIV	60	150	0	40
530/565 (HEX)	HCOV	40	150	0	40
585/630 (ROX)	MPV	80	150	0	40
630/665 (Cy5)	HADV	80	150	0	40
680/715 (Cy5.5)	EIC	80	150	0	35

Table 6. PCR settings for snap-in 4.

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 the "Color compensation" field enter the following parameters (Tables 7 and 8).

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	4	0	0	0
	530/565	1	-	0	0	0
	585/630	0	0	-	1	0
	630/665	0	0	3	-	18
	680/715	0	0	0	1.5	-

Table 7. "Color compensation" parameters for snap-in 2.

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	0	0	0	0
	530/565	0	-	0	0	0
	585/630	0	0	-	3	0
	630/665	0	0	5	-	19
	680/715	0	0	0	3	-

Table 8. "Color compensation" parameters for snap-in 4.

In the "Melt Settings" tabs no action is needed, it is not applicable to this product.

In the "Test Steps" tab:

11) Enter the step name (up to twenty characters) and set the following parameters to define each step of the PCR protocol: "Profile Type", "Cycles", "Time" and "Temperature" and select the "Detect" field to define the detection step (Table 9). Click the "Add" button to add a new step and repeat until all the steps needed are defined.

Note: The "Type" field must be empty.

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

Table 9. PCR protocol for snap-in 2 and snap-in 4.

In the "Result Logic" tab:

12) In the "Target" field name your target: i.e. SARS (up to seven alphanumeric characters). Repeat steps 12-15 for each target (i.e. SARS, FLUB, FLUA and RSV for snap-in 2 or HPIV, HCOV, MPV and HADV for snap-in 4) following the tables specific for the target being defined.

    Note: Select the snap-in 2 (green) in the "Master Mix" drop down menu to stablish the result logic for the first reaction mix and the snap-in 4 (blue) for the second one. The target names must be different for snap-in 2 and snap-in 4.

13) Click the "Analyze" checkbox to include the desired wavelengths (PCR channels) in the target result analysis (Tables 10-13 for snap-in 2 and tables 14-17 for snap-in 4).

First master mix (*Respiratory Virus Mix I* reaction tube): Snap-in 2 (green)

Wavelength	Alias	Type	Analyze
475/520	SARS	PCR	✓
680/715	EIC	PCR	✓

Table 10. PCR channels selection in the "Result logic" tab for SARS (SARS-CoV-2) target.

Wavelength	Alias	Type	Analyze
530/565	FLUB	PCR	✓
680/715	EIC	PCR	✓

Table 11. PCR channels selection in the "Result logic" tab for FLUB (Influenza B) target.

Wavelength	Alias	Type	Analyze
585/630	FLUA	PCR	✓
680/715	EIC	PCR	✓

Table 12. PCR channels selection in the "Result logic" tab for FLUA (Influenza A) target.

Wavelength	Alias	Type	Analyze
630/665	RSV	PCR	✓
680/715	EIC	PCR	✓

Table 13. PCR channels selection in the "Result logic" tab for RSV (Respiratory Syncytial Virus types A and B) target.

Second master mix (*Respiratory Virus Mix II* reaction tube): Snap-in 4 (blue)

Wavelength	Alias	Type	Analyze
475/520	HPIV	PCR	✓
680/715	EIC	PCR	✓

Table 14. PCR channels selection in the "Result logic" tab for HPIV (Parainfluenza types 1, 2, 3 and 4) target.

Wavelength	Alias	Type	Analyze
530/565	HCOV	PCR	✓
680/715	EIC	PCR	✓

Table 15. PCR channels selection in the "Result logic" tab for HCOV (Coronavirus 229E, NL63, HKU1 and OC43) target.

Wavelength	Alias	Type	Analyze
585/630	MPV	PCR	✓
680/715	EIC	PCR	✓

Table 16. PCR channels selection in the "Result logic" tab for MPV (Metapneumovirus) target.

Wavelength	Alias	Type	Analyze
630/665	HADV	PCR	✓
680/715	EIC	PCR	✓

Table 17. PCR channels selection in the "Result logic" tab for HADV (Adenovirus) target.

14) Click the "Edit Logic" button.

15) In the "Edit Logic" window all the combinations of result types are listed. For each row, in the "Result" drop down menu select the result that is called when the conditions in that row are met, following tables 18-21 for snap-in 2 and tables 22-25 for snap-in 4.

First master mix (*Respiratory Virus Mix* / reaction tube): Snap-in 2 (green)

Result	SARS (475/520)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 18. List of the combination of result types and Result logic for SARS (SARS-CoV-2) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	FLUB (530/565)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 19. List of the combination of result types and Result logic for FLUB (Influenza B) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	FLUA (585/630)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 20. List of the combination of result types and Result logic for FLUA (Influenza A) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	RSV (630/665)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 21. List of the combination of result types and Result logic for RSV (Respiratory Syncytial Virus types A and B) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Note: According to the Ct Max previously defined (table 5):

i. The result type for SARS (475/520), FLUB (530/565), FLUA (585/630) or RSV (630/665) channels is considered "Valid" when the Ct value obtained is ≤40; and "Invalid" when the Ct value obtained is >40.

ii. The result type for EIC (680/715) channel is considered Valid" when the Ct value obtained is ≤35 "; and "Invalid" when the Ct value obtained is >35.

Second master mix (*Respiratory Virus Mix II* reaction tube): Snap-in 4

Result	HPIV (475/520)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 22. List of the combination of result types and Result logic for HPIV (Parainfluenza types 1, 2, 3 and 4) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	HCOV (530/565)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 23. List of the combination of result types and Result logic for HCOV (Coronavirus 229E, NL63, HKU1 and OC43) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	MPV (585/630)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 24. List of the combination of result types and Result logic for MPV (Metapneumovirus) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Result	HADV (630/665)	EIC (680/715)
POS	Valid	Valid
UNR	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

Table 25. List of the combination of result types and Result logic for HADV (Adenovirus) target. Available results are POS (Positive), NEG (Negative) and UNR (Unresolved).

Note: According to the Ct Max previously defined (table 6):

i. The result type for HPIV (475/520), HCOV (530/565), MPV (585/630) or HADV (630/665) channels is considered "Valid" when the Ct value obtained is ≤40; and "Invalid" when the Ct value obtained is >40.

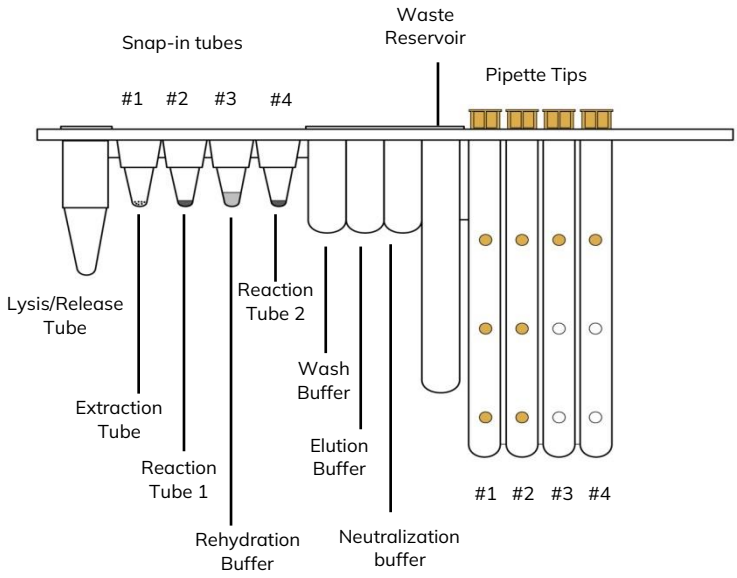
ii. The result type for EIC (680/715) channel is considered Valid" when the Ct value obtained is ≤35 "; and "Invalid" when the Ct value obtained is >35.

16) Click the "Save" button to save the test.

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.
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).
    a. Remove excess air, and close the pouch with the zip seal.
    b. 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.
5) Determine and separate the appropriate number of *Respiratory Virus Mix II* reaction tubes (1M 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 “Worklist” tab on the “Run” screen of the BD MAX™ System software v4.50A or higher.
2) In the “Test” drop down menu, select the desired test: i.e. VIASURE Resp virus (if not already created see Section 8.3.1).
3) In the “Kit Lot Number” drop down menu, select the appropriate lot number for the kit (found on the outer box of extraction kit used) (optional).

   Note: Lot numbers must be defined in the “Inventory” display before they can be selected here.
4) Enter the Sample Buffer Tube identification number into the “Sample tube” field, either by scanning the barcode with the scanner or by manual entry.
5) Fill the “Patient ID” and/or “Accession” field and click the Tab or Enter key. Continue until all Sample Buffer Tubes barcodes 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” to begin the procedure.

8.3.4. BD MAX™ results report

1) From the menu bar, click the “Results” button.
2) Either double click on your run in the list or press the “view” button.

3) The “Print” and "Export" buttons at the bottom of the screen will be enabled.

To print the results:

1. Click on “Print” button.
2. In the “Print” preview window of the run report select: “Run Details”, “Test Details” and “Plots”.
3. Click on “Print” to print the report or click “Export” to export a PDF of the report to a USB.

To export the results:

1. Click on “export” button to transfer the report (PDF and CSV file) to a USB.
2. When the export is complete, the success/fail icon appears in the “Results Export” window.

9. Result interpretation

For a detailed description on how to analyse 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 5). 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 there was no Ct value calculated by the software or the Ct value calculated is below the specified threshold or above the stablished Ct Max (cutoff).

- Any other Ct value should be interpreted in correlation with the amplification curve and according to the result logic defined, following the interpretation guidelines outlined in Tables 26 and 27.

Check Endogenous 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 analysed using the following tables:

First master mix (*Respiratory Virus Mix* / reaction tube): Snap-in 2				
SARS-CoV-2 (name target: SARS)	**Influenza B (name target: FLUB)**	**Influenza A (name target: FLUA)**	**Respiratory Syncytial virus (name target: RSV)**	**Interpretation for patient’s individual samples**
POS	POS	POS	POS	**SARS-CoV-2, Influenza B, Influenza A and RSV RNA detected**

POS	POS	POS	NEG	SARS-CoV-2, Influenza B and Influenza A RNA Detected, RSV RNA Not Detected
POS	POS	NEG	POS	SARS-CoV-2, Influenza B and RSV RNA Detected, Influenza A RNA Not Detected
POS	NEG	POS	POS	SARS-CoV-2, Influenza A and RSV RNA Detected, Influenza B RNA Not Detected
NEG	POS	POS	POS	Influenza B, Influenza A and RSV RNA detected, SARS-CoV-2 RNA Not Detected
POS	POS	NEG	NEG	SARS-CoV-2 and Influenza B RNA detected, Influenza A and RSV RNA Not Detected
POS	NEG	POS	NEG	SARS-CoV-2 and Influenza A RNA detected, Influenza B and RSV RNA Not Detected
POS	NEG	NEG	POS	SARS-CoV-2 and RSV RNA detected, Influenza B and Influenza A RNA Not Detected
NEG	POS	POS	NEG	Influenza B and Influenza A RNA detected, SARS-CoV-2 and RSV RNA Not Detected
NEG	POS	NEG	POS	Influenza B and RSV RNA detected, SARS-CoV-2 and Influenza A RNA Not Detected
NEG	NEG	POS	POS	Influenza A and RSV RNA detected, SARS-CoV-2 and Influenza B RNA Not Detected
POS	NEG	NEG	NEG	SARS-CoV-2 RNA detected, Influenza B, Influenza A and RSV RNA Not Detected
NEG	POS	NEG	NEG	Influenza B RNA detected, SARS-CoV-2, Influenza A and RSV RNA Not Detected
NEG	NEG	POS	NEG	Influenza A RNA detected, SARS-CoV-2, Influenza B and RSV RNA Not Detected
NEG	NEG	NEG	POS	RSV RNA detected, SARS-CoV-2, Influenza B and Influenza A RNA Not Detected
NEG	NEG	NEG	NEG	RNA target not detected
UNR	UNR	UNR	UNR	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.[1]
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.[2]
INC	INC	INC	INC	Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run. [2]

Table 26. Sample interpretation.

1 The Endogenous Internal Control (EIC) must show an amplification signal with Ct value ≤ 35 to be considered. If there is an absence of signal for EIC or Ct value > 35, the result is considered as Unresolved (UNR) and retesting is required. Check the result report and Ct values of the selected targets and carry out appropriate action considering the following:

I. When targeted genes results are invalid (Ct > 40, which is shown by the software as a "-1" result), it is required to repeat the assay from the primary sample preparing the Sample Buffer Tube (SBT) again if enough sample volume is available. Follow the laboratory guidelines and/or microbiology laboratory policy manuals.

II. When targeted genes results are valid (Ct ≤ 40), it is possible to see no amplification or amplification from EIC with a Ct value >35 (which is shown by the software as a "-1" result) when testing highly concentrated samples, due to a preferential amplification of target-specific nucleic acids. If considered necessary, dilute these samples 1/10, prepare the Sample Buffer Tube (SBT) again and repeat testing. Follow the laboratory guidelines and/or microbiology laboratory policy manuals.

NOTE: Nasopharyngeal swabs can be kept without transfer to the SBT for up to 2 days if stored at 25°C or up to 7 days if stored at 4°C.

2 Indeterminate (IND) or Incomplete (INC) results may be obtained due to a system failure and retesting is required. Refer to the BD MAX™ System's User Manual for interpretation of warning and error codes.

Second master mix (*Respiratory Virus Mix II* reaction tube): Snap-in 4				
Parainfluenza (name target: HPIV)	**Coronavirus (name target: HCOV)**	**Metapneumovirus (name target: MPV)**	**Adenovirus (name target: HADV)**	**Interpretation for patient's individual samples**
POS	POS	POS	POS	**Parainfluenza, coronavirus, metapneumovirus and adenovirus RNA/DNA detected**
POS	POS	POS	NEG	**Parainfluenza, coronavirus and metapneumovirus RNA Detected, adenovirus DNA Not Detected**
POS	POS	NEG	POS	**Parainfluenza, coronavirus and adenovirus RNA/DNA Detected, metapneumovirus RNA Not Detected**
POS	NEG	POS	POS	**Parainfluenza, metapneumovirus and adenovirus RNA/DNA Detected, coronavirus RNA Not Detected**
NEG	POS	POS	POS	**Coronavirus, metapneumovirus and adenovirus RNA/DNA Detected, Parainfluenza RNA Not Detected**
POS	POS	NEG	NEG	**Parainfluenza and coronavirus RNA Detected, metapneumovirus and adenovirus RNA/DNA Not Detected**
POS	NEG	POS	NEG	**Parainfluenza and metapneumovirus RNA Detected, coronavirus and adenovirus RNA/DNA Not Detected**
POS	NEG	NEG	POS	**Parainfluenza and adenovirus RNA/DNA Detected, coronavirus and metapneumovirus RNA Not Detected**
NEG	POS	POS	NEG	**Coronavirus and metapneumovirus RNA Detected, parainfluenza and adenovirus RNA/DNA Not Detected**
NEG	POS	NEG	POS	**Coronavirus and adenovirus RNA/DNA Detected, parainfluenza and metapneumovirus RNA Not Detected**
NEG	NEG	POS	POS	**Metapneumovirus and adenovirus RNA/DNA Detected, parainfluenza and coronavirus RNA Not Detected**

POS	NEG	NEG	NEG	**Parainfluenza RNA Detected, coronavirus, metapneumovirus and adenovirus RNA/DNA Not Detected**
NEG	POS	NEG	NEG	**Coronavirus RNA Detected, parainfluenza, metapneumovirus and adenovirus RNA/DNA Not Detected**
NEG	NEG	POS	NEG	**Metapneumovirus RNA Detected, parainfluenza, coronavirus and adenovirus RNA/DNA Not Detected**
NEG	NEG	NEG	POS	**Adenovirus DNA Detected, parainfluenza, coronavirus and metapneumovirus RNA Not Detected**
NEG	NEG	NEG	NEG	**RNA target not detected**
UNR	UNR	UNR	UNR	**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.[1]**
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.[2]**
INC	INC	INC	INC	**Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run. [2]**

Table 27. Sample interpretation.

1 The Endogenous Internal Control (EIC) must show an amplification signal with Ct value ≤ 35 to be considered. If there is an absence of signal for EIC or Ct value > 35, the result is considered as Unresolved (UNR) and retesting is required. Check the result report and Ct values of the selected targets and carry out appropriate action considering the following:

I. When targeted genes results are invalid (Ct > 40, which is shown by the software as a "-1" result), it is required to repeat the assay from the primary sample preparing the Sample Buffer Tube (SBT) again if enough sample volume is available. Follow the laboratory guidelines and/or microbiology laboratory policy manuals.

II. When targeted genes results are valid (Ct ≤ 40), it is possible to see no amplification or amplification from EIC with a Ct value >35 (which is shown by the software as a "-1" result) when testing highly concentrated samples, due to a preferential amplification of target-specific nucleic acids. If considered necessary, dilute these samples 1/10, prepare the Sample Buffer Tube (SBT) again and repeat testing. Follow the laboratory guidelines and/or microbiology laboratory policy manuals.

NOTE: Nasopharyngeal swabs can be kept without transfer to the SBT for up to 2 days if stored at 25°C or up to 7 days if stored at 4°C.

2 Indeterminate (IND) or Incomplete (INC) results may be obtained due to a system failure and retesting is required. Refer to the BD MAX™ System's User Manual for interpretation of warning and error codes.

Note: When using external controls, they should yield the following expected results; negative for ENC and positive for EPC (known positive specimens are expected to be positive only for the microorganism(s) present in the specimen). An ENC that yields a positive test result is indicative of a contamination event or specimen handling failure. An EPC that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique. When an external control failure occurs, 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 PCR 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 only 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.
- The quality of the test depends on the quality of the sample; nucleic acid must be properly extracted from clinical samples.
- This test is a qualitative test and does not provide quantitative values or indicate the number of organisms present. It is not possible to correlate the Ct values obtained by PCR with the concentration of the sample as they depend on the thermal cycler used and the run itself.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- Please note the intended measurement range of the assay, as samples with concentrations above or below this range may give erroneous results.
- There is a possibility of false positive results due to cross-contamination by SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and adenovirus samples containing high concentrations of target RNA/DNA 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, *M* gene (matrix protein (M1)) for Influenza A/B, the *HA* gene for Influenza A H1N1 subtype, *N* gene of

RSV (types A and B), *HN* gen of parainfluenza (types 1, 2 and 3), *F* gene of parainfluenza (type 4), *N* gen of coronavirus (229E, NL63, HKU1 and OC43), *F* gen of metapneumovirus and *hexon* gen of adenovirus, used in VIASURE *Respiratory Virus Extended Mix* 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/DNA extraction).
    - Degradation of the RNA/DNA 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 A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and/or adenovirus strains.
    - A viral load in the specimen below the limit of detection for the assay.
    - The presence of RT-qPCR inhibitors or other types of interfering substances. The impact of vaccines, some antiviral therapeutics, antibiotics, chemotherapeutics, immunosuppressant drugs or antifungals used to prevent the infection or used during the treatment of the infection has not been evaluated.
    - The effect of interfering substances has only been evaluated for those indicated in section 12.7.1 (study of interfering substances) of this instruction for use. Interference was observed when testing fluticasone (1.26E-06 mg/ml) and nicotine (3.00E-02 mg/ml) in both *Respiratory Virus Mix I* reaction tube and *Respiratory Virus Mix II* reaction tube. Please, see this section to check the most common endogenous and exogenous substances that induce a total or partial interference of RT-qPCR reaction. Other substances not indicated in this part could lead to erroneous results.
    - Failure to follow instructions for use and the assay procedure.
- 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 targeted virus sequences.
- Negative results do not preclude the presence of SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and/or adenovirus RNA/DNA in a clinical specimen 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 these microorganisms have not been determined. The collection of multiple specimens (types and time points) from the same patient may be necessary to detect the pathogen.

- It is possible that the detection of some circulating strains from 2019 belonging to the Victoria Lineage of Influenza B could be compromised due to point mutations in these new strains. Only the inclusivity of Influenza B was tested for variants containing mutations C54T, C55T and C120T (reference sequence NC002210.1).
- If diagnostic tests for other respiratory illnesses are negative and clinical observations, patient history and epidemiological information suggest that SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and/or adenovirus infection is possible, then a false negative result should be considered, and a re-testing of the patient should be discussed.
- Fluorescence values may vary due to multiple factors such as: PCR equipment (even being the same model), extraction system, type of sample, previous treatment of the sample, etc... among others.
- The device includes primers and probes specifically detecting the Influenza A H1N1pdm09 strain. Pandemic influenza strain cannot be excluded, and additional tests should be considered in case of a positive influenza result.
- Positive and negative predictive values are highly dependent on prevalence in all *in vitro* diagnostic tests. VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System performance may vary depending on the prevalence and population tested.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *Respiratory Virus Extended Mix* 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 Extended Mix* 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. Besides, the use of external controls (EPC and ENC) allows to confirm the assay performance. External controls are not used by the BD MAX™ System for the purpose of result interpretation but considered as a sample. External Positive Control (EPC) is intended to monitor a potential failure of the assay reagents, while External Negative Control (ENC) is intended to detect environmental or reagent contamination by target nucleic acids.

12. Analytical performance characteristics

12.1. Analytical linearity

The linearity of the assay was determined and confirmed by testing a series of ten-fold dilutions of nasopharyngeal swab samples containing a known concentration of specific and synthetic RNA/DNA belonging to of SARS-CoV-2, influenza A virus, influenza B virus, human respiratory syncytial virus, parainfluenza virus, coronavirus, metapneumovirus and adenovirus (ranging from 2E+07 to 2E+00 copies per µl). Example of the amplification plots resulting from an assay is included below:

Figure 2. Dilution series of SARS-CoV-2 (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (475/520 (FAM) channel).

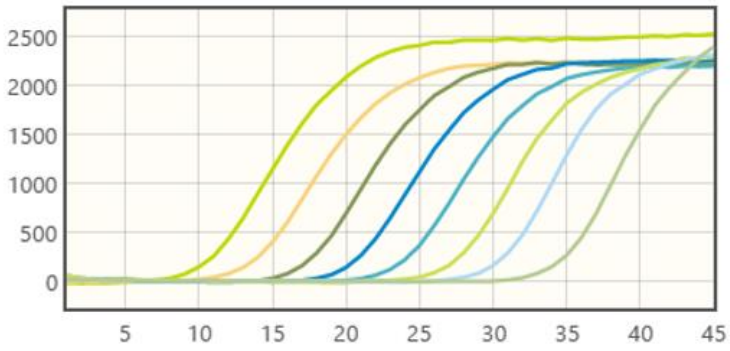


Figure 3. Dilution series of Influenza B (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (530/565 (HEX) channel).

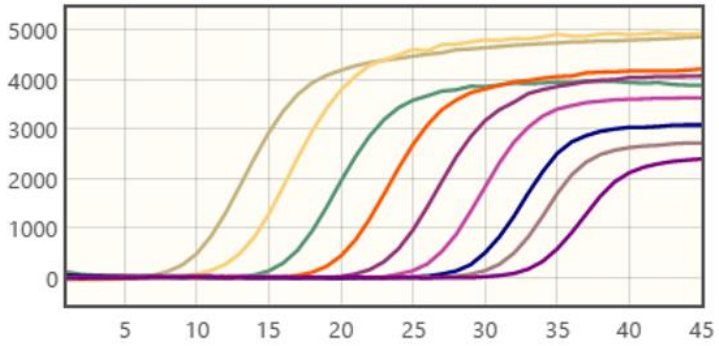


Figure 4. Dilution series of Influenza A (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (585/630 (ROX) channel).

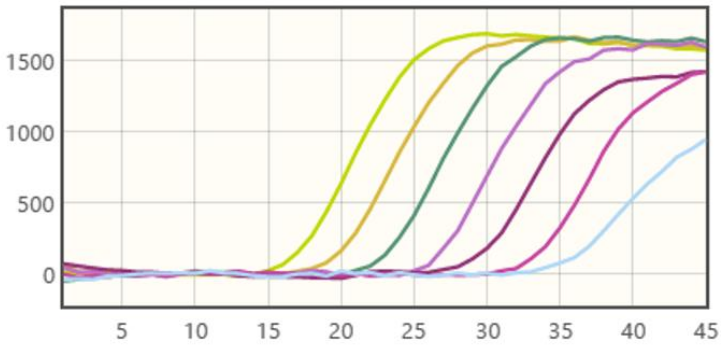


Figure 5. Dilution series of RSV (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (630/665 (Cy5) channel).

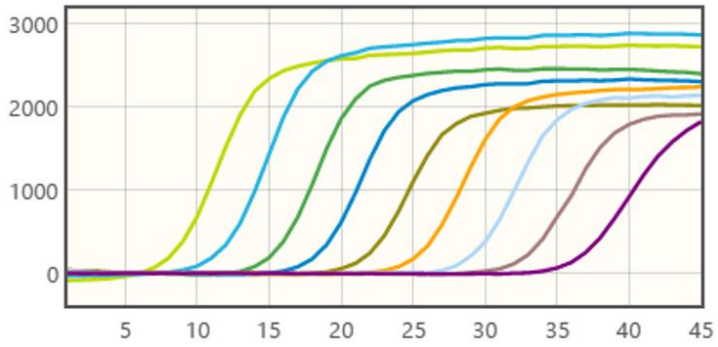


Figure 6. Dilution series of parainfluenza (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (475/520 (FAM) channel).

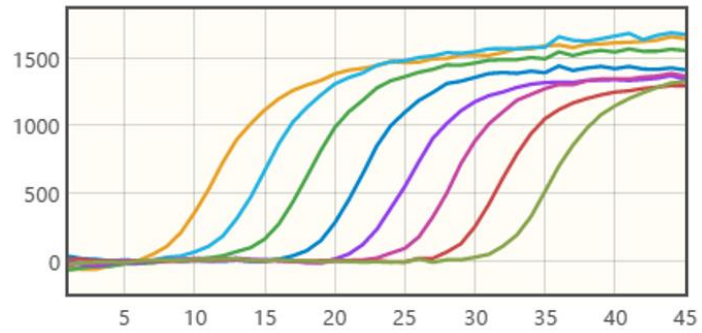


Figure 7. Dilution series of coronavirus (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (530/565 (HEX) channel).

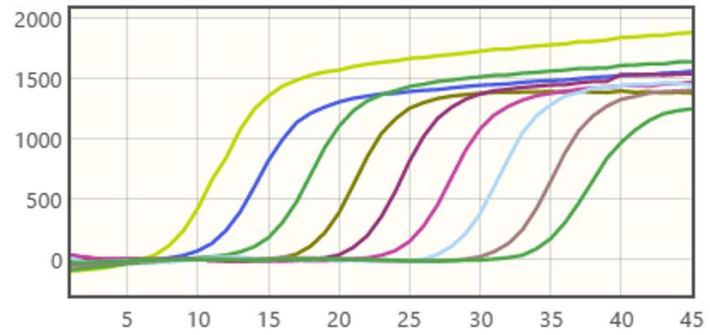


Figure 8. Dilution series of metapneumovirus (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (585/630 (ROX) channel).

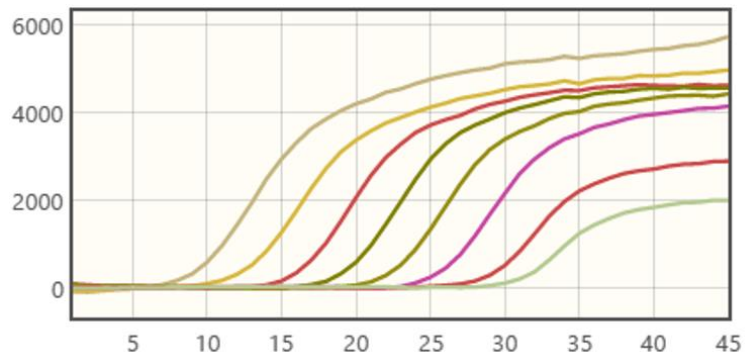
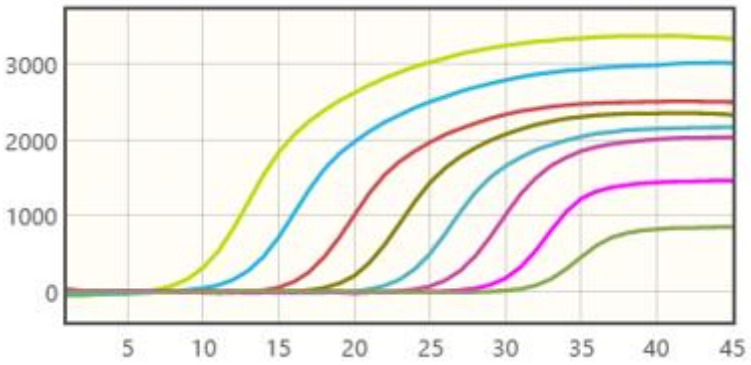


Figure 9. Dilution series of adenovirus (2E+07 to 2E+00 copies/µl) template run on the BD MAX™ System (630/665 (Cy5) channel).



12.2. Analytical sensitivity. Limit of Detection (LoD)

Analytical sensitivity or limit of detection (LoD) of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System was analysed with three lots using negative nasopharyngeal samples collected in BD™ Universal Viral Transport System spiked with the reference strains or synthetic RNA detailed in the following table:

Virus	Strain/synthetic RNA	External reference
SARS-CoV-2	Heat-inactivated SARS-CoV-2 strain 2019-n-CoV/USA-WA1/2020	VR-1986HK
Influenza A	Influenza A virus (H1N1) strain A/PR/8/34	VR-95PQ
Influenza B	Influenza B virus (Yamagata Lineage) strain B/Florida/4/2006	VR-1804PQ
RSV A	Human respiratory syncytial virus A Strain Long	VR-26PQ
RSV B	Human Respiratory Syncytial Virus B strain 9320	FR-293
Parainfluenza virus type 1	Human parainfluenza virus 1 strain C35	VR-94
Parainfluenza virus type 2	Human parainfluenza virus 2 strain Greer	VR-92
Parainfluenza virus type 3	Human parainfluenza virus 3 strain C 243	VR-93
Parainfluenza virus type 4	Human parainfluenza virus 4b strain CH 19503	VR-1377
Coronavirus OC43	Betacoronavirus 1	VR-1558
Coronavirus 229E	Human coronavirus 229E	VR-740
Coronavirus NL63	Human Coronavirus, Strain NL63	FR-304
Coronavirus HKU1	Quantitative synthetic Human coronavirus HKU1 RNA	ATCC-VR3262SD
Metapneumovirus	Human Metapneumovirus 8 (hMPV-8) Type B2 Culture Fluid (Heat Inactivated)	0810159CF
Adenovirus	Human adenovirus 1 strain Adenoid 71	VR-1

Table 28. Reference strains and synthetic RNA used for VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System performance assays (LoD assay).

Besides, in the case of SARS-CoV-2 target, the LoD (IU/µL) was analysed using 1st WHO International Standard SARS-CoV-2 RNA (NIBSC code 20/146). The LoD results obtained for VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System are detailed in the following table:

Nasopharyngeal swab			
Respiratory Virus Mix I **reaction tube**			
SARS	**FLUA**	**FLUB**	**RSV**
4.5E+00 IU/µL	6.7E-01 copies/µL	7.29E+00 copies/µL	1.35E+00 copies/µL RSV A 2.52E+01 TCID50/ml RSV B
Respiratory virus Mix II **reaction tube**			
HPIV	**HCOV**	**MPV**	**HADV**
5.33E+00 TCID50/ml HPIV 1 4.80E+00 TCID50/ml HPIV 2 9.00E+02 TCID50/ml HPIV 3 1.44E+01 TCID50/ml HPIV 4	4.80E-02 TCID50/ml HCOV OC43 1.60E-01 TCID50/ml HCOV 229E 4.80E-03 TCID50/ml HCOV NL63 6.00E+00 copies/µL HCOV HKU1	5.10E-02 TCID50/ml	6.00E+00 TCID50/ml

Table 29. Limit of detection results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. IU = International Unit, TCID50 = Median Tissue Culture Infection Dose.

12.3. Measuring range

The measuring range of the assay was determined by testing a series of ten-fold dilutions containing a known concentration of specific and synthetic NA belonging to SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and adenovirus. Results allowed to confirm the correct targets detection from 2E+07 to 2E+00 copies/µL, except for HCoV-HKU1 whose measuring range goes from 2E+06 to 2E+00 copies/µl.

In conclusion, measuring range of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System was successfully determined, ensuring reliable, accurate and reproducible results across a wide spectrum of viral loads, affirming its utility in various clinical diagnostic scenarios.

12.4. Accuracy

12.4.1. Trueness (Veracity)

The veracity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System was evaluated by testing reference material listed below spiked in negative nasopharyngeal samples collected in BD™ Universal Viral Transport System.

1. **Synthetic DNA fragments**
    - Synthetic DNA fragment for *ORF1* gene of SARS-CoV-2: NCOXPC, FAM channel.
    - Synthetic DNA fragment for *N* gene of SARS-CoV-2: NCOXPC, FAM channel.
    - Synthetic DNA fragment for *M1* gene of Flu A: YIAXPC, ROX channel.
    - Synthetic DNA fragment for *HA* gene of Flu A: HNVXPC, ROX channel.
    - Synthetic DNA fragment for *M1* gene of Flu B: YIBXPC, HEX channel.

- Synthetic DNA fragment for *N* gene of RSV A: RSAXPC, Cy5 channel.
- Synthetic DNA fragment for *N* gene of RSV B: RSBXPC, Cy5 channel.
- Synthetic DNA fragment for *HN* gene of parainfluenza 1: PIXPC, FAM channel.
- Synthetic DNA fragment for *HN* gene of parainfluenza 2: PIXPC, FAM channel.
- Synthetic DNA fragment for *HN* gene of parainfluenza 3: PIXPC, FAM channel.
- Synthetic DNA fragment for *F* gene of parainfluenza 4: PIXPC, FAM channel.
- Synthetic DNA fragment for *N* gene of coronavirus OC43: CORXPC, HEX channel.
- Synthetic DNA fragment for *N* gene of coronavirus-229E: CORXPC, HEX channel.
- Synthetic DNA fragment for *N* gene of coronavirus-NL63: CORXPC, HEX channel.
- Synthetic DNA fragment for *N* gene of coronavirus HKU1: CORXPC, HEX channel.
- Synthetic DNA fragment for *F* gene of metapneumovirus: MPVXPC, ROX channel.
- Synthetic DNA fragment for *hexon* gene of adenovirus: ADVXPC, Cy5 channel.

All the synthetic DNA fragments were detected correctly in the appropriate channel.

2. The American Type Culture Collection ("ATCC®")

External Reference	Microorganism	Product name	Variety	Result
VR-1986HK	SARS-CoV-2	Heat-inactivated SARS-CoV-2	2019-nCoV/USA-WA1/2020	Detected
VR-3276SD	SARS-CoV-2	Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N	N/A	Detected
VR-1986D	SARS-CoV-2	Genomic RNA from 2019 Novel Coronavirus	SARS-Related Coronavirus 2, Isolate USA-WA1/2020	Detected
VR-95PQ	Flu A	Influenza A virus (H1N1), Purified	A/PR/8/34	Detected
VR-1804PQ	Flu B	Influenza B virus (Yamagata Lineage), Purified	B/Florida/4/2006	Detected
VR-26PQ	RSV-A	Human respiratory syncytial virus, High titer	Strain Long	Detected
VR-94	Parainfluenza virus 1	Human parainfluenza virus 1 (HPIV-1)	Strain C35	Detected
VR-92	Parainfluenza virus 2	Human parainfluenza virus 2 (HPIV-2)	Strain Greer	Detected
VR-93	Parainfluenza virus 3	Human parainfluenza virus 3 (HPIV-3)	Strain C 243	Detected
VR-1377	Parainfluenza virus 4	Human parainfluenza virus 4 (HPIV-4b)	Strain CH 19503	Detected
VR-1558	Coronavirus OC43	Betacoronavirus 1	Strain OC43	Detected

VR-740	Coronavirus 229E	Human coronavirus 229E	Strain 229E	Detected
VR-3262SD	Coronavirus HKU1	Quantitative Synthetic Human Coronavirus Strain HKU1 RNA	Strain HKU1	Detected
VR-3263SD	Coronavirus NL63	Quantitative Synthetic Human coronavirus Strain NL63 RNA	Strain NL63	Detected
VR-3250SD	Metapneumovirus	Synthetic Human metapneumovirus RNA	N/A	Detected
VR-1	Adenovirus	Human adenovirus 1	Strain Adenoid 71	Detected
VR-6	Adenovirus	Human Adenovirus 6	Type 6 (Species C) Strain Tonsil 99	Detected
VR-16	Adenovirus	Human Adenovirus 15	Type 15 (Species D) Strain 305 [955, CH. 38]	Detected
VR-3343	Adenovirus	Human adenovirus 31	Type 31 (Species A) Strain 1315/63	Detected

Table 30. Reference material from the American Type Culture Collection (ATTC®).

All the strains from the ATCC were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤35.

3. The International Reagent Resource (IRR™)

External Reference	Microorganism	Product name	Variety	Result
FR-293	RSV-B	Human Respiratory Syncytial Virus B	Strain 9320	Detected
FR-304	Coronavirus NL63	Human Coronavirus, Strain NL63	Strain NL63	Detected
FR-1	Flu A	Influenza A virus	A/Brisbane/59/2007 (H1N1)	Detected
FR-3	Flu A	Influenza A virus	A/South Dakota/6/2007 (H1N1)	Detected
FR-5	Flu A	Influenza A virus	A/Hawaii/31/2007 (H1N1)	Detected
FR-6	Flu A	Influenza A virus	A/Qatar/1123/2007 (H1N1)	Detected
FR-7	Flu A	Influenza A virus	A/Cambodia/0371/2007 (H1N1)	Detected
FR-8	Flu A	Influenza A virus	A/Brisbane/10/2007 (H3N2)	Detected
FR-12	Flu A	Influenza A virus	A/Taiwan/760/2007 (H3N2)	Detected
FR-13	Flu A	Influenza A virus	A/Texas/71/2007 (H3N2)	Detected

FR-27	Flu A	Influenza A virus	A/Brisbane/10/2007 IVR-147 (H3N2)	Detected
FR-28	Flu A	Influenza A virus	A/Brisbane/59/2007 IVR-148 (H1N1)	Detected
FR-29	Flu A	Influenza A virus	A/South Dakota/6/2007 X-173 (H1N1)	Detected
FR-201	Flu A	Influenza A virus	A/California/07/2009 (H1N1)pdm09	Detected
FR-202	Flu A	Influenza A virus	A/California/08/2009 (H1N1)pdm09	Detected
FR-203	Flu A	Influenza A virus	A/New York/18/2009 (H1N1)pdm09	Detected
FR-245	Flu A	Influenza A virus	A/Mexico/4108/2009 (H1N1)pdm09	Detected
FR-246	Flu A	Influenza A virus	A/California/07/2009 NYMC X-179A (H1N1)pdm09	Detected
FR-16	Flu B	Influenza B virus	B/Pennsylvania/7/2007 (Yamagata Lineage)	Detected
FR-17	Flu B	Influenza B virus	B/Santiago/4364/2007 (Yamagata Lineage)	Detected
FR-18	Flu B	Influenza B virus	B/Brisbane/3/2007 (Yamagata Lineage)	Detected
FR-19	Flu B	Influenza B virus	B/Pennsylvania/5/2007 (Victoria Lineage)	Detected
FR-20	Flu B	Influenza B virus	B/Victoria/304/2006 (Victoria Lineage)	Detected
FR-183	Flu B	Influenza B virus	B/Bangladesh/3333/2007 (Yamagata Lineage)	Detected
FR-294	RSV A	Human Respiratory Syncytial Virus	Strain A-2	Detected

Table 31. Reference material from the International Reagent Resource (IRR™).

All the strains from the IRR were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤35.

4. National Institute for Biological Standards and Control (NIBSC)

External Reference	Microorganism	Product name	Variety	Result
20/146	SARS-CoV-2	First WHO International Standard for SARS-CoV-2 RNA	England/02/2020 isolate	Detected
20/110	SARS-CoV-2	2019 novel coronavirus (SARS-CoV-2) Working Reagent for NAT	N/A	Detected
19/304	SARS-CoV-2	Research Reagent for SARS-CoV-2 RNA	N/A	Detected
08/176	Parainfluenza virus 1	Parainfluenza Virus Serotype 1	N/A	Detected

08/178	Parainfluenza virus 2	Parainfluenza Virus Serotype 2	N/A	Detected
08/180	Parainfluenza virus 4	Parainfluenza Virus Serotype 1	N/A	Detected
08/320	Metapneumovirus	Human Metapneumovirus Working Reagent for NAT	N/A	Detected
16/324	Adenovirus	First WHO International Standard for Human Adenovirus DNA	Type 2	Detected

Table 32. Reference material from the National Institute for Biological Standards and Control (NIBSC).

All the strains from the NIBSC were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤35.

5. BEI Resources

External Reference	Microorganism	Product name	Variety	Result
NR-52287	SARS-CoV-2	SARS-Related Coronavirus 2	Isolate USA-WA1/2020, Gamma-Irradiated	Detected
NR-28530	RSV	Human Respiratory Syncytial Virus, A2000/3-4	Strain A2000/3-4	Detected
NR-22227	Metapneumovirus	Human metapneumovirus	TN/83-1211	Detected

Table 33. Reference material from the BEI Resources.

All the strains from BEI Resources were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤35.

6. Control material

External Reference	Microorganism	Product name	Variety	Result
Fluarix Tetra 2022/2023	Flu A/Flu B	Influenza vaccine Fluarix Tetra 2022/2023	Flu A/Victoria/2570/2019 Flu A/Darwin/6/2021 Flu B/Austria/1359417/2021 Flu B/Phuket/3073/2013	Detected
Fluarix Tetra 2023/2024	Flu A/Flu B	Influenza vaccine Fluarix Tetra 2023/2024	Flu A/Victoria/4897/2022 Flu A/Darwin/6/2021 Flu B/Austria/1359417/2021 Flu B/Phuket/3073/2013	Detected
102019	SARS-CoV-2	Synthetic SARS-CoV-2 RNA Control 1	SARS-CoV-2 isolate Australia/VIC01/2020	Detected
102024	SARS-CoV-2	Synthetic SARS-CoV-2 RNA Control 2	SARS-CoV-2 isolate Wuhan-Hu-1	Detected
103907	SARS-CoV-2	Synthetic SARS-CoV-2 RNA Control 14	UK variant (B.1.1.7_710528)	Detected
103909	SARS-CoV-2	Synthetic SARS-CoV-2 RNA Control 15	UK variant (B.1.1.7_601443)	Detected
104043	SARS-CoV-2	Synthetic SARS-CoV-2 RNA Control 16	South Africa variant	Detected

104044	SARS-CoV-2	Synthetic SARS-CoV-2 RNA Control 17	Japan/Brazil variant	Detected
0505-0129	SARS-CoV-2	Accuplex™ SARS-CoV-2 Verification Panel	N/A	Detected
MBC139-R	SARS-CoV-2	AMPLIRUN® SARS-CoV-2 B.1.351 RNA CONTROL	B.1.351 lineage	Detected
MBTC030-R	SARS-CoV-2	AMPLIRUN® TOTAL SARS-CoV-2 CONTROL	B.1.351 lineage	Detected
MBTC031-R	SARS-CoV-2, Flu A, Flu B and RSV	AMPLIRUN® TOTAL SARS-CoV-2/FLUA/FLUB/RSV CONTROL	Influenza A H3N2 (A/Perth/16/2009), Influenza B (B/Brisbane/60/2008), RSV (9320)	Detected
MBC144-R	MPV	AMPLIRUN® METAPNEUMOVIRUS RNA CONTROL	Lineage B1	Detected
SCV2_24C1B-01	SARS-CoV-2	SARS-CoV-2 Delta variant	Delta variant B.1.617.2	Detected
SCV2_23C1D-01	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage XBB	Detected
SCV2_23C1D-02	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BQ1.1	Detected
SCV2_23C1D-03	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.2.75	Detected
SCV2_23C1D-04	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.5	Detected
SCV2_23C1D-05	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BQ.1	Detected
SCV2_23C1B-01	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.4	Detected
SCV2_23C1B-03	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.5	Detected
SCV2_23C1B-04	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.4	Detected
SCV2_23C1B-05	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.2	Detected
SCV2_23C1C-01	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.2.75	Detected
SCV2_23C1C-02	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BQ.1	Detected
SCV2_23C1C-03	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BQ1.1	Detected
SCV2_23C1C-04	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage XBB	Detected
SCV2_23C1C-05	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.2	Detected
SCV2_23C1A-01	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.5	Detected
SCV2_23C1A-02	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.2	Detected

SCV2_23C1A-03	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.5	Detected
SCV2_23C1A-05	SARS-CoV-2	SARS-CoV-2 Omicron variant	Sublineage BA.4	Detected
ID3-09 2023	RSV	Influenza A virus	Strain A/Cambodia/e0826360/2020 (H3N2)	Detected
359043	RSV	Human Respiratory Syncytial Virus, B	N/A	Detected
PINFRNA101S-06	Parainfluenza virus type 2	PIV-2	N/A	Detected
PINFRNA22S-02	Parainfluenza virus type 3	PIV-3	N/A	Detected
CVRNA22S-04	Coronavirus	Coronavirus HKU	Strain HKU	Detected
0810110CF	Adenovirus	Adenovirus Culture Fluid (Heat Inactivated) Type 2	Type 2 (Species C)	Detected
0810062CFHI	Adenovirus	Adenovirus Culture Fluid (Heat Inactivated) Type 3	Type 3 (Species B)	Detected
0810070CFHI	Adenovirus	Adenovirus Culture Fluid (Heat Inactivated) Type 4	Type 4 (Species E)	Detected
0810020CF	Adenovirus	Adenovirus Culture Fluid (Heat Inactivated) Type 5	Type 5 (Species C)	Detected
0810021CFHI	Adenovirus	Adenovirus Culture Fluid (Heat Inactivated) Type 7A	Type 7A (Species B)	Detected
0810119CFHI	Adenovirus	Adenovirus Culture Fluid (Heat Inactivated) Type 37	Type 37	Detected
0810084CFHI	Adenovirus	Adenovirus Type 40 Culture Fluid (Heat Inactivated) Strain Dugan	Type 40, strain Dugan	Detected
0810085CFHI	Adenovirus	Adenovirus Type 41 Culture Fluid (Heat Inactivated) Strain Tak	Type 41 (Species F), strain Tak	Detected
0810159CF	MPV	Human Metapneumovirus 8	Type B2	Detected

Table 34. Control material for SARS-CoV-2, Influenza A, Influenza B, RSV, parainfluenza, coronavirus, metapneumovirus and adenovirus.

All the strains were correctly detected in the appropriate channel and the EIC showed amplification with a Ct value ≤35.

7. External Quality Assessment (EQA) Prorgammes

A total of 85 samples containing SARS-Cov-2, influenza A, influenza B, RSV, parainfluenza virus, coronavirus, metapneumovirus and/or adenovirus, among other nontarget microorganisms, were analysed from QCMD, INSTAND, CAP and RCPA programmes, showing high concordance.

12.4.2. Precision

To determine the precision of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System, intra-assay (repeatability), and inter-assay, inter-batch and inter-equipment assays (reproducibility) were performed with nasopharyngeal swab collected with BD™ Universal Viral Transport System spiked with the reference strains mentioned in table 28 for the representative targets selected for each fluorescence channel: SARS-CoV-2, influenza A, Influenza B, RSV B, parainfluenza virus type 3, coronavirus OC43, metapneumovirus and adenovirus.

Intra-assay

The intra-assay was tested by analysing six replicates of all samples in the same run using VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. A summary of results is shown in the tables below.

Respiratory Virus Mix I reaction tube					
Target	**Samples**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
SARS-CoV-2	2xLoD	475/520 (FAM)	30.30	0.31	1.02
	5xLoD	475/520 (FAM)	29.15	0.45	1.53
	Negative control	475/520 (FAM)	Neg	N/A	N/A
Influenza B	2xLoD	530/565 (HEX)	32.98	0.53	1.62
	5xLoD	530/565 (HEX)	31.32	0.52	1.68
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Influenza A	2xLoD	585/630 (ROX)	33.45	1.35	4.03
	5xLoD	585/630 (ROX)	31.37	0.67	2.15
	Negative control	585/630 (ROX)	Neg	N/A	N/A
RSV	2xLoD	630/665 (CY5)	32.18	1.49	4.64
	5xLoD	630/665 (CY5)	31.02	0.56	1.79
	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	26.17	0.38	1.46
	5xLoD	680/715 (CY5.5)	25.97	0.50	1.93
	Negative control	680/715 (CY5.5)	26.37	0.91	3.46

Table 35. Intra-assay results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

Respiratory Virus Mix II reaction tube					
Target	**Samples**	**Channel**	**Ct (x̄)**	**σ**	**CV %**
Parainfluenza virus	2xLoD	475/520 (FAM)	32.13	0.56	1.76
	5xLoD	475/520 (FAM)	29.97	1.16	3.88
	Negative control	475/520 (FAM)	Neg	N/A	N/A
Coronavirus	2xLoD	530/565 (HEX)	34.23	0.73	2.14
	5xLoD	530/565 (HEX)	32.03	0.85	2.65
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Metapneumovirus	2xLoD	585/630 (ROX)	31.53	0.66	2.09
	5xLoD	585/630 (ROX)	29.63	0.77	2.61
	Negative control	585/630 (ROX)	Neg	N/A	N/A
Adenovirus	2xLoD	630/665 (CY5)	34.35	0.10	0.31
	5xLoD	630/665 (CY5)	33.55	1.39	4.15
	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	25.72	0.53	2.08
	5xLoD	680/715 (CY5.5)	25.57	0.61	2.39
	Negative control	680/715 (CY5.5)	25.63	0.23	0.91

Table 36. Intra-assay results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. (x̄) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

Inter-assay

The inter-assay was tested by testing four replicates of the different samples on three different days by three different operators, using the VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. A summary of results is shown in the tables below.

Respiratory Virus Mix I reaction tube					
Target	**Samples**	**Channel**	**Ct (x̄)**	**σ**	**CV %**
SARS-CoV-2	2xLoD	475/520 (FAM)	30.33	0.38	1.27
	5xLoD	475/520 (FAM)	28.93	0.28	0.97
	Negative control	475/520 (FAM)	Neg	N/A	N/A
Influenza B	2xLoD	530/565 (HEX)	32.61	0.66	2.03
	5xLoD	530/565 (HEX)	31.55	0.46	1.45
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Influenza A	2xLoD	585/630 (ROX)	32.94	0.99	2.99
	5xLoD	585/630 (ROX)	31.95	0.76	2.37
	Negative control	585/630 (ROX)	Neg	N/A	N/A
RSV	2xLoD	630/665 (CY5)	32.80	0.74	2.27
	5xLoD	630/665 (CY5)	31.67	0.53	1.68

	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	25.90	0.25	0.95
	5xLoD	680/715 (CY5.5)	25.99	0.40	1.55
	Negative control	680/715 (CY5.5)	25.84	0.31	1.21

Table 37. Inter-assay results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

Respiratory Virus Mix II reaction tube					
Target	**Samples**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Parainfluenza virus	2xLoD	475/520 (FAM)	31.86	0.71	2.22
	5xLoD	475/520 (FAM)	30.18	0.82	2.72
	Negative control	475/520 (FAM)	Neg	N/A	N/A
Coronavirus	2xLoD	530/565 (HEX)	33.45	0.76	2.27
	5xLoD	530/565 (HEX)	31.56	0.96	3.04
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Metapneumovirus	2xLoD	585/630 (ROX)	30.79	0.93	3.01
	5xLoD	585/630 (ROX)	29.68	0.88	2.97
	Negative control	585/630 (ROX)	Neg	N/A	N/A
Adenovirus	2xLoD	630/665 (CY5)	35.08	1.62	4.61
	5xLoD	630/665 (CY5)	33.20	0.77	2.33
	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	25.49	0.31	1.21
	5xLoD	680/715 (CY5.5)	25.65	0.33	1.30
	Negative control	680/715 (CY5.5)	25.52	0.31	1.21

Table 38. Inter-assay results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

Inter-batch

The inter-batch values were determined with six replicates of the different samples by using three batches of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. A summary of results is shown in the tables below.

Respiratory Virus Mix I reaction tube					
Target	**Samples**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
SARS-CoV-2	2xLoD	475/520 (FAM)	29.07	0.45	1.53
	5xLoD	475/520 (FAM)	29.14	0.59	2.01
	Negative control	475/520 (FAM)	Neg	N/A	N/A

Influenza B	2xLoD	530/565 (HEX)	32.58	0.64	1.95
	5xLoD	530/565 (HEX)	31.01	1.03	3.31
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Influenza A	2xLoD	585/630 (ROX)	32.75	1.7	5.33
	5xLoD	585/630 (ROX)	31.61	1.14	3.62
	Negative control	585/630 (ROX)	Neg	N/A	N/A
RSV	2xLoD	630/665 (CY5)	31.72	1.90	5.99
	5xLoD	630/665 (CY5)	30.21	1.35	4.46
	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	25.40	0.31	1.23
	5xLoD	680/715 (CY5.5)	25.61	0.53	2.06
	Negative control	680/715 (CY5.5)	25.54	0.37	1.45

Table 39. Inter-batch results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

Respiratory Virus Mix II reaction tube					
Target	**Samples**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Parainfluenza virus	2xLoD	475/520 (FAM)	31.45	1.20	3.82
	5xLoD	475/520 (FAM)	29.53	0.59	1.99
	Negative control	475/520 (FAM)	Neg	N/A	N/A
Coronavirus	2xLoD	530/565 (HEX)	32.35	0.71	2.20
	5xLoD	530/565 (HEX)	30.18	1.33	4.39
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Metapneumovirus	2xLoD	585/630 (ROX)	31.66	1.00	3.16
	5xLoD	585/630 (ROX)	29.16	1.25	4.29
	Negative control	585/630 (ROX)	Neg	N/A	N/A
Adenovirus	2xLoD	630/665 (CY5)	33.23	0.70	2.12
	5xLoD	630/665 (CY5)	32.83	1.06	3.23
	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	24.82	0.43	1.73
	5xLoD	680/715 (CY5.5)	25.57	0.42	1.64
	Negative control	680/715 (CY5.5)	25.03	0.36	1.43

Table 40. Inter-batch results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

Inter-equipment

The inter-equipment values were determined with six replicates of the same samples used for intra-assay, inter-assay and inter-batch, using the VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. These assays were run at two laboratory sites with two different BD MAX™ System. A summary of results is shown in the tables below.

Respiratory Virus Mix I reaction tube					
Target	**Samples**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
SARS-CoV-2	2xLoD	475/520 (FAM)	30.30	0.29	0.96
	5xLoD	475/520 (FAM)	28.93	0.24	0.82
	Negative control	475/520 (FAM)	Neg	N/A	N/A
Influenza B	2xLoD	530/565 (HEX)	32.70	0.42	1.29
	5xLoD	530/565 (HEX)	31.26	0.45	1.43
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Influenza A	2xLoD	585/630 (ROX)	33.33	1.47	4.42
	5xLoD	585/630 (ROX)	31.52	0.61	1.92
	Negative control	585/630 (ROX)	Neg	N/A	N/A
RSV	2xLoD	630/665 (CY5)	32.80	0.76	2.33
	5xLoD	630/665 (CY5)	30.88	1.30	4.20
	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	35.97	0.32	1.24
	5xLoD	680/715 (CY5.5)	25.71	0.33	1.28
	Negative control	680/715 (CY5.5)	25.96	0.37	1.44

Table 41. Inter-equipment results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

Respiratory Virus Mix II reaction tube					
Target	**Samples**	**Channel**	**Ct ($\bar{x}$)**	**σ**	**CV %**
Parainfluenza virus	2xLoD	475/520 (FAM)	31.95	0.83	2.61
	5xLoD	475/520 (FAM)	29.79	0.95	3.18
	Negative control	475/520 (FAM)	Neg	N/A	N/A
Coronavirus	2xLoD	530/565 (HEX)	34.26	0.74	2.15
	5xLoD	530/565 (HEX)	31.95	0.61	1.91
	Negative control	530/565 (HEX)	Neg	N/A	N/A
Metapneumovirus	2xLoD	585/630 (ROX)	31.59	0.50	1.58
	5xLoD	585/630 (ROX)	29.51	1.03	3.48
	Negative control	585/630 (ROX)	Neg	N/A	N/A
Adenovirus	2xLoD	630/665 (CY5)	34.14	0.72	2.12

	5xLoD	630/665 (CY5)	32.31	0.36	1.13
	Negative control	630/665 (CY5)	Neg	N/A	N/A
EIC	2xLoD	680/715 (CY5.5)	25.35	0.22	0.85
	5xLoD	680/715 (CY5.5)	25.10	0.22	0.87
	Negative control	680/715 (CY5.5)	25.73	0.28	1.11

Table 42. Inter-equipment results of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. (Ct) = threshold cycle. ($\bar{x}$) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation, Neg = negative, N/A = not applicable.

In conclusion, the precision study confirmed the reliable performance and consistency of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System.

12.5. Carry-over

The Robustness (Carry-over parameter) was analysed according to the common specifications for certain class D IVD devices in accordance with Regulation (EU) 2017/746; in particular, the Annex XIII, which lays down common specifications for devices intended for detection or quantification of markers of SARS-CoV-2 virus infection. These recommendations only applied to the SARS-CoV-2 virus target, the rest of the targets of *Respiratory Virus Mix I* reaction tube were not analysed in this assay. However, Parainfluenza virus type 3 was also analysed as a target of the *Respiratory Virus Mix II* reaction tube to perform the assay with both Master Mixes.

The carry-over contamination rate was determined by testing 60 replicates of negative nasopharyngeal swab specimens, and 60 replicates of a SARS-CoV-2 sample RNA positive with a high titter of viral SARS-CoV-2 RNA (First WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146), at 1E+02 IU/mL) and a Human parainfluenza virus type 3 sample RNA positive with a high titter of viral HPIV 3 RNA (Human parainfluenza virus 3 (ATCC Code: VR-93™), at 1E+05 TCID50/ml). In total, five runs of positive and negative samples were performed using an assay method based on the checkerboard layout, which allows samples to be arrange alternately.

For *Respiratory Virus Mix I* reaction tube, 60 out of 60 positive samples were correctly detected, and 59 out of 60 negative samples gave a negative result, giving an agreement rate of 98.33%. For *Respiratory Virus Mix II* reaction tube, all positive samples were correctly detected, and all negative samples gave a negative result. According to the results of both reaction tubes the cross-contamination rate was almost 0%.

12.6. Whole-system failure rate

The Robustness (Whole system failure rate parameter) was analysed according to the indications of the common specifications for devices intended for detection or quantification of markers of SARS-CoV-2 virus infection. As these recommendations only applied to the SARS-CoV-2 virus target, the rest of the targets were not analysed in this assay.

To demonstrate that SARS-CoV-2 RNA low-positive specimens are detected by VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System, 119 negative nasopharyngeal swab specimens, spiked with First WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) at a virus concentration equivalent to three times its LoD (3xLoD) were tested with VIASURE assay.

The results of the study indicate that all replicates were reactive for SARS-CoV-2 RNA target, corresponding to an agreement rate of 100%. In conclusion, the system-wide failure rate is 0%.

12.7. Analytical specificity and reactivity

The analytical specificity and analytical reactivity were evaluated for the VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System *in silico* and experimentally, using different starting material such as certified reference strains, certified reference RNA/DNAs and material from the EQAs programmes.

12.7.1. Analytical Specificity

Analytical specificity is the assay's ability to detect the intended target. There are two components to be considered for analytical specificity: Cross-reactivity and Interference. Cross-reactivity may occur when genetically related sequences are present in a patient specimen, while interference may happen if the presence of specific substances potentially present in the sample matrixes affects the performance of the RT-qPCR.

Cross-reactivity *in silico* analysis

The Cross-reactivity was assessed by using reference sequences of the pathogens from NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/), and an in-house bioinformatic analysis software. BLAST analysis over each primer and probe over the NCBI Genbank Nucleotide Database and an in-house bioinformatic analysis were performed.

Aligned sequences with a percentage of alignment less than 80% of homology were considered unlikely to be detected. Results obtained demonstrated that **all analyzed sequences were below 80% of homology with SARS-CoV-2, Influenza A, Influenza B and RSV (types A and B) primers and probe sets included in *Respiratory Virus Mix I* reaction tube; parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63 and HKU1), metapneumovirus and adenovirus primers and probe sets included in *Respiratory Virus Mix II* reaction tube**.

In the case of Coronavirus OC43, the following results were obtained:

Coronavirus OC43

BLAST analysis filtered by coronavirus OC43 (excluding OC43 Taxonomy ID: 31631) shows high homology between the primers and probes and betacoronavirus 1 from strains of bovine, buffalo, oryx, giraffe, camel, canine, equine, porcine, rabbit, Sable antelope, Sambar deer, Tapir, Water deer, Waterbuck, Watusi, White-tailed deer, Yak. However, these viruses have not been identified in humans and are not considered zoonotic viruses for the time being, so they do not interfere in the detection of coronavirus OC43.

Therefore, none of the sequences analyzed, including those showing a homology higher than 80%, could affect the correct detection of coronavirus OC43.

In conclusion, the SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), human coronavirus (229E, NL63, HKU1 and OC43), metapneumovirus and adenovirus target designs of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System should not cause false positives in detecting these microorganisms when other organisms are present.

Analytical specificity wet testing

Cross-reactivity wet testing

The cross-reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System was confirmed by testing a panel of different microorganisms associated with respiratory infections spiked in nasopharyngeal swab collected with BD™ Universal Viral Transport System. When possible and the concentration data available, the virus and bacteria interfering were evaluated at medically relevant levels (usually 1E+05 – 1E+06 cfu (colony-forming unit)/ml for bacteria and 1E+04 – 1E+05 pfu (plaque-forming unit)/ml for viruses). No cross-reactivity was detected between any of the following microorganisms tested, except for the targeted microorganisms.

Cross-reactivity testing					
Adenovirus Type 15 (Species D), strain 35 [955, CH.38]	+/-	Influenza A virus A/Cambodia/e0826360/2020 (H3N2)	+/-	SARS-CoV-2	+/-
Adenovirus Type 2, species C	+/-	Influenza A Virus A/Hawaii/31/2007 (H1N1)	+/-	SARS-CoV-2	+/-
Adenovirus Type 3, species B	+/-	Influenza A Virus A/Mexico/4108/2009 (H1N1)pdm09	+/-	SARS-CoV-2 B.1.1.7_601443, UK variant	+/-
Adenovirus Type 31 (Species A) Strain 1315/63	+/-	Influenza A Virus A/New York/18/2009 (H1N1)pdm09	+/-	SARS-CoV-2 B.1.1.7_710528, UK variant	+/-
Adenovirus Type 37	+/-	Influenza A Virus A/Qatar/1123/2007 (H1N1)	+/-	SARS-CoV-2 B.1.351	+/-
Adenovirus Type 4, species E	+/-	Influenza A Virus A/South Dakota/6/2007 (H1N1)	+/-	SARS-CoV-2 Coronavirus 2 strain 2019-nCoV/USA-WA1/2020	+/-
Adenovirus Type 40, strain Dugan	+/-	Influenza A Virus A/South Dakota/6/2007 X-173 (H1N1)	+/-	SARS-CoV-2 Delta variant B.1.617.2	+/-
Adenovirus Type 41 (Species F), strain Tak	+/-	Influenza A Virus A/Taiwan/760/2007 (H3N2)	+/-	SARS-CoV-2 Omicron variant, sublineage BA.2	+/-
Adenovirus Type 5, species C	+/-	Influenza A Virus A/Texas/71/2007 (H3N2)	+/-	SARS-CoV-2 Omicron Variant, sublineage BA.2	+/-

Cross-reactivity testing					
Adenovirus Type 6 (Species C), strain Tonsil 99	+/-	Influenza B Virus B/Bangladesh/3333/2007 (Yamagata Lineage)	+/-	SARS-CoV-2 Omicron Variant, sublineage BA.2	+/-
Adenovirus Type 7A, species B	+/-	Influenza B Virus B/Brisbane/3/2007 (Yamagata Lineage)	+/-	SARS-CoV-2 Omicron variant, sublineage BA.2.75	+/-
Adenovirus Human Adenovirus DNA Type 2	+/-	Influenza B Virus B/Pennsylvania/5/2007 (Victoria Lineage)	+/-	SARS-CoV-2 Omicron Variant, sublineage BA.2.75	+/-
Bordetella holmesii	-	Influenza B Virus B/Pennsylvania/7/2007 (Yamagata Lineage)	+/-	SARS-CoV-2 Omicron Variant, sublineage BA.4	+/-
Bordetella parapertussis	-	Influenza B Virus B/Santiago/4364/2007 (Yamagata Lineage)	+/-	SARS-CoV-2 Omicron variant, sublineage BA.4	+/-
Bordetella pertussis	-	Influenza B Virus B/Victoria/304/2006 (Victoria Lineage)	+/-	SARS-CoV-2 Omicron Variant, sublineage BA.4	+/-
Bordetella pertussis Type strain	-	*Klebsiella pneumoniae* subsp. Pneumoniae Strain PCI 602	-	SARS-CoV-2 Omicron variant, sublineage BA.5	+/-
Candida albicans	-	Legionella Pneumophila Sg1 (ST47)	-	SARS-CoV-2 Omicron Variant, sublineage BA.5	+/-
Chlamydophila pneumoniae Strain CM-1	-	*Legionella pneumophila* Sg1 (ST62)	-	SARS-CoV-2 Omicron Variant, sublineage BA.5	+/-
Coronavirus HKU	+/-	*Legionella pneumophila* subsp. Pneumophila Strain Philadelphia-1	-	SARS-CoV-2 Omicron Variant, sublineage BA.5	+/-
Coronavirus Strain NL63	+/-	MERS-CoV Strain Florida/USA-2_Saudi Arabia_2014	-	SARS-CoV-2 Omicron Variant, sublineage BQ.1	+/-
Enterovirus D58 US/MO/14-18949	-	*Moraxella catarrhalis* Strain 59632	-	SARS-CoV-2 Omicron Variant, sublineage BQ.1	+/-
Haemophilus influenzae	-	*Mycoplasma pneumoniae*	-	SARS-CoV-2 Omicron variant, sublineage BQ1.1	+/-
Haemophilus influenzae	-	*Mycoplasma pneumoniae* Strain PI 1428	-	SARS-CoV-2 Omicron Variant, sublineage BQ1.1	+/-
Haemophilus influenzae Strain L-378	-	Parainfluenza virus type 2	+/-	SARS-CoV-2 Omicron variant, sublineage XBB	+/-
Human rinovirus 17 Strain 33342	-	Parainfluenza virus type 3	- +/-	SARS-CoV-2 Omicron Variant, sublineage XBB	+/-
Influenza A Virus A/Brisbane/10/2007 (H3N2)	+/-	*Pneumocystis jirovecii*	-	SARS-CoV-2 SARS-CoV-2 isolate Australia/VIC01/2020	+/-
Influenza A Virus A/Brisbane/10/2007 IVR-147 (H3N2)	+/-	*Pseudomonas aeruginosa* Strain RH 815	-	SARS-CoV-2 SARS-CoV-2 isolate Wuhan-Hu-1	+/-
Influenza A Virus A/Brisbane/59/2007 (H1N1)	+/-	RSV A 2000/3-4	+/-	SARS-CoV-2	+/-
Influenza A Virus A/Brisbane/59/2007 IVR-148 (H1N1)	+/-	RSV Strain A-2	+/-	*Staphylococcus epidermis* Strain PCI 1200	-
Influenza A Virus A/California/07/2009 (H1N1)pdm09	+/-	RSV Type B	+/-	*Streptococcus pneumoniae*	-
Influenza A Virus A/California/07/2009 NYMC X-179A (H1N1)pdm09	+/-	SARS-CoV-1 Strain Frankfurt 1	+/-	*Streptococcus pneumoniae* Strain [CIP 104225]	-
Influenza A Virus A/Cambodia/0371/2007 (H1N1)	+/-				

Table 43. Reference pathogenic microorganisms included in the cross-reactivity assay. The +/- result refers to the positive or negative result obtained in the different channels depending on the target detected. In case a microorganism tested is one of the targets detected by the device, a positive result is obtained in their corresponding channel, but a negative result is obtained in the other channels.

In conclusion, the results from cross-reactivity assays indicate high specificity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for the detection of the targeted microorganisms, thereby minimizing the risk of false-positive results. Since no non-specific amplifications were observed with other related microorganisms, this suggests that the device is capable of accurately distinguishing the targets.

Study of co-infection

A study of coinfection was conducted using reference strains detailed in table 28 for the representative targets selected for each fluorescence channel: SARS-CoV-2, influenza A, Influenza B, RSV B, parainfluenza virus type 3, coronavirus OC43, metapneumovirus and adenovirus; at different concentrations, to confirm that the presence of any of them, independently of the concentration, does not alter the detection between them. Nine nasopharyngeal samples spiked with the reference material, one target at low concentration (3xLoD) and the other targets at a very high concentration, usually 1E+04 – 1E+05 units/ml, if possible, were analysed.

The results confirm that the detection of the microorganisms targeted is not altered when analysed with VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System in coinfection at different concentrations.

Study of interfering microbial agents

A study of interfering microbial agents was performed to analyse the potential interfering microbial agents for VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. A panel of different microorganisms associated to respiratory diseases was tested in presence of SARS-CoV-2, influenza A and B viruses, RSV B, parainfluenza virus type 3, coronavirus OC43, metapneumovirus and adenovirus (reference strains detailed in table 28) at 3xLoD. When possible and the concentration data available, the virus and bacteria interfering were evaluated at medically relevant levels (usually 1E+05 – 1E+06 CFU (colony-forming unit)/ml for bacteria and 1E+04 – 1E+05 pfu (plaque-forming unit)/ml for viruses). Each point analysis was conducted twice per specimen.

Positive Matrix Control and Negative Matrix Control (PMC and NMC, respectively) are included as controls of the test. PMC corresponds to the negative nasopharyngeal matrix spiked with specific target strains without any interfering microbial agent, whereas NMC corresponds to the negative nasopharyngeal matrix without any interfering microbial agent.

Respiratory Virus Mix I reaction tube and *Respiratory Virus Mix II* reaction tube		
Microorganism Name	Concentration tested	Result
PMC	N/A	N.I
NMC	N/A	N.I
Human rhinovirus 17	1.60E+04 TCID50/ml	N.I
Enterovirus D58	4.00E+04 TCID50/ml	N.I
MERS-CoV	3.55E+03 TCID50/ml	N.I
Chlamydophila pneumoniae	3.16E+04 TCID50/ml	N.I
Streptococcus pneumoniae	1.80E+03 CFU/µl	N.I
Mycoplasma pneumoniae	1.00E+05 CFU/ml	N.I
Candida albicans	4.18E+06 CFU/ml	N.I
Staphylococcus epidermidis	3.60E+06 CFU/ml	N.I
SARS-CoV 1	5.20E+02 cop/ml	N.I
Bordetella pertussis	1.20E+05 CFU/ml	N.I
Bordetella holmesii	4.10E+04 CFU/ml	N.I
Bordetella parapertussis	1.20E+05 CFU/ml	N.I
Klebsiella pneumoniae	3.65E+04 CFU/µl	N.I
Moraxella catarrhalis	1.00E+06 CFU/ml	N.I
Legionella pneumohila subsp. pneumophila	5.60E+04 CFU/µl	N.I
Haemophilus influenzae	5.20E+03 CFU/µl	N.I
Pseudomonas aeruginosa	4.90E+06 CFU/ml	N.I
Pneumocystis jirovecii	1.00E+03 cop/µl	N.I

Table 44. Interfering microbial agents assay. N.I. = No interference.

In conclusion, no interference was observed in the detection the nucleic acid targeted with any of the microorganisms tested.

Study of interfering substances

A study of interfering substances was performed to test the potential interfering effect of endogenous and exogenous substances on VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. A total of twenty potentially interfering substances were added to the negative nasopharyngeal matrix enriched with the reference strains detailed in Table 28 for SARS-CoV-2, influenza A Influenza B, RSV B, parainfluenza virus type 3, coronavirus OC43, metapneumovirus and adenovirus and evaluated with six replicates.

Positive Matrix Control and Negative Matrix Control (PMC and NMC, respectively) are included as controls of the test. PMC corresponds to the negative nasopharyngeal matrix spiked with specific target strains without interfering substance, whereas NMC corresponds to the negative nasopharyngeal matrix without interfering substance nor added microorganisms/reference material. The following results were obtained:

Respiratory Virus Mix I reaction tube and *Respiratory Virus Mix II* reaction tube		
Substance name	**Concentration tested**	**Result**
PMC	N/A	N.I.
NMC	N/A	N.I.
Oseltamivir	3.99E-04 mg/ml	N.I.
Zanamivir	3.30 mg/ml	N.I.
Azithromycin	1.10E-02 mg/ml	N.I.
Mupirocin	1.50E-03 mg/ml	N.I.
Tobramycin	3.30E-02 mg/ml	N.I.
Albumin	1.00E+01mg/ml	N.I.
Genomic DNA	3.50E-03 mg/ml	N.I.
Human mucus	1.00 % (v/v)	N.I.
Mucin	2.50E+00 mg/ml	N.I.
Triglycerides	1.50E+01 mg/ml	N.I.
Whole Blood	1.00 % (v/v)	N.I.
Carbocysteine	5.00E+00 mg/ml	N.I.
N-acetylcysteine	1.50E-01 mg/ml	N.I.
Phenylephrine	3.00E-05 mg/ml	N.I.
Fluticasone	1.26E-06 mg/ml	I
	3.15E-07 mg/ml	N.I.
Galphimia glauca, luffa operculata	1.25E+01 mg/ml	N.I.
Oxymetazoline hydrochloride	1.00E-01 mg/ml	N.I.
Sodium chloride	9.00E-01 mg/ml	N.I.
Nicotine	3.00E-02 mg/ml	I
	7.50E-04 mg/ml	N.I.
Benzocaine	3.00E+00 mg/ml	N.I.

Table 45. Potential interference substances. N.I: No reportable interfere, I: Interference.

Different potentially interfering substances, both endogenous and exogenous, were tested on VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System. Interference was observed when testing fluticasone (1.26E-06 mg/ml) and nicotine (3.00E-02 mg/ml) in both *Respiratory Virus Mix I* reaction tube and *Respiratory Virus Mix II* reaction tube and, a dilution 1/4 was performed to assess that this interference effect is not observed at lower concentrations. The results obtained lead to conclude that, at the final concentrations tested, no interference of any the evaluated substances is observed at the concentrations tested.

12.7.2. Analytical reactivity

Analytical reactivity can be defined as the percentage of target microbial strains or DNA/RNA samples that give the correct positive result. Analytical reactivity was studied *in silico* and by wet analyses.

Analytical reactivity *in silico* analysis

The Analytical Reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System was assessed by using publicly available nucleotide sequence database as NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/), Global Initiative on Sharing All SARS-CoV-2 Data (GISAID EpiCoV database (https://www.gisaid.org/)), Global Initiative on Sharing All Influenza Data (GISAID EpiFlu database (https://www.gisaid.org/)), Global Initiative on Sharing All RSV Data (GISAID EpiRSV database (https://www.gisaid.org/)), and an in-house bioinformatic analysis software, in order to demonstrate that the target genes can be correctly detected by the device under study. *In silico* analysis of the primers and probe design was performed through alignment against sequences available the database nucleotide collection (nr/nt). The results obtained after the analysis of sequences included are shown in the following table:

Microorganism	Gene	% sequences experimentally detected without mismatches	% sequences experimentally detected with mismatches	Number of aligned sequences
SARS-CoV-2	*N* gene, region N1 and region N2	98.08%	-	22,404
Influenza A	*HA gene + M1* gene	0.88%	32.92%	99,326
Influenza B	*M1* gene	21.63%	70.58%	24,369
RSV A	*N* gene	2.32%	80.83%	4,002
RSV B	*N* gene	2.61%	80.11%	4,172
Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4	*HN* gene *HN* gene *HN* gene *F* gene	19.23%	-	1451
Coronavirus OC43	*N* gene	76.32%	-	380
Coronavirus 229E	*N* gene	0.00%*	-	266
Coronavirus NL63	*N* gene	48.12%	-	293
Coronavirus HKU1	*N* gene	46.51%	-	215
Metapneumovirus	*F* gene	93.7%	-	2,144
Adenovirus A	*Hexon* gene	98.7%	-	154
Adenovirus B	*Hexon* gene	98.75%	-	718
Adenovirus C	*Hexon* gene	96.17%	-	392
Adenovirus D	*Hexon* gene	97.74%	-	310
Adenovirus E	*Hexon* gene	89.53%	-	172
Adenovirus F	*Hexon* gene	96.40%	-	250
Adenovirus G	*Hexon* gene	90.48%	-	21

Table 46. Analytical reactivity *in silico* assay.

*Most of the coronavirus 229E sequences from NCBI GenBank included in the *in silico* analysis have 1 or 2 mismatches that do not affect the correct detection of the target. Besides, the synthetic DNA of Coronavirus 229E used during the analytical validation of the product shows 100% homology with the set of primers and probes, thus demonstrating experimentally its correct detection.

To sum up, the inclusivity analysis shown a correct detection of SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), coronavirus (NL63, 229E, HKU1 and OC43), metapneumovirus and adenovirus with the VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System.

Analytical reactivity wet testing

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for SARS-CoV-2 was evaluated against the RNA from the following strains spiked in nasopharyngeal swab collected with BD™ Universal Viral Transport System, showing positive results:

SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (NR-52287), Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N (VR-3276SD), Genomic RNA from 2019 Novel Coronavirus (VR-1986D), 2019 novel coronavirus (SARS-CoV-2) Working Reagent for Nucleic Acid Amplification Testing (NAT) (NIBSC 20/110), Research Reagent for SARS-CoV-2 RNA (NIBSC 19/304), Twist Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1): SARS-CoV-2 isolate Australia/VIC01/2020 (Twist Bioscience 102019), Twist Synthetic SARS-CoV-2 RNA Control 2 (MN908947.3): SARS-CoV-2 isolate Wuhan-Hu-1 (Twist Bioscience 102024), Twist Synthetic SARS-CoV-2 RNA Control 14 (B.1.1.7_710528), UK variant (Twist Bioscience 103907), Twist Synthetic SARS-CoV-2 RNA Control 15 (B.1.1.7_601443), UK variant (Twist Bioscience 103909), Twist Synthetic SARS-CoV-2 RNA Control 16 (EPI_ISL_678597), South Africa variant (Twist Bioscience 104043), Twist Synthetic SARS-CoV-2 RNA Control 17 (EPI_ISL_792683), Japan/Brazil variant (Twist Bioscience 104044), Accuplex™ SARS-CoV-2 Verification Panel (SeraCare c0505-0129), AMPLIRUN® SARS-CoV-2 B.1.351 RNA CONTROL (MBC139-R), AMPLIRUN® TOTAL SARS-CoV-2 CONTROL (SWAB) (MBTC030-R), AMPLIRUN TOTAL SARS-CoV-2-FluA-FluB-RSV CONTROL (MBTC031-R), SARS-CoV-2 Omicron Varian, sublineage BA.5 (SCV2_23C1A-01), SARS-CoV-2 Omicron Variant, sublineage BA.2 (SCV2_23C1A-02), SARS-CoV-2 Omicron Variant, sublineage BA.5 (SCV2_23C1A-03), SARS-CoV-2 Omicron Variante, sublineage BA.4 (SCV2_23C1A-05), SARS-CoV-2 Omicron Variant, sublineage BA.4 (SCV2_23C1B-01), SARS-CoV-2 Omicron Variant, sublineage BA.5 (SCV2_23C1B-03, SARS-CoV-2 Omicron variant, sublineage BA.4 (SCV2_23C1B-04), SARS-CoV-2 Omicron variant, sublineage BA.2 (SCV2_23C1B-05), SARS-CoV-2 Delta Variant B.1.617.2 (SCV2_24C1B-01), SARS-CoV-2 Omicron Variant, sublineage BA.2.75 (SCV2_23C1C-01), SARS-CoV-2 Omicron Variant, sublineage BQ.1 (SCV2_23C1C-02), SARS-CoV-2 Omicron Variant, sublineage BQ1.1 (SCV2_23C1C-03), SARS-CoV-2 Omicron Variant, sublineage XBB (SCV2_23C1C-04), SARS-CoV-2 Omicron Variant, sublineage BA.2 (SCV2_23C1C-05), SARS-CoV-2 Omicron variant, sublineage XBB (SCV2_23C1D-01), SARS-CoV-2 Omicron variant, sublineage BQ1.1 (SCV2_23C1D-02), SARS-CoV-2 Omicron variant, sublineage BA.2.75 (SCV2_23C1D-03), SARS-CoV-2 Omicron Variant, sublineage BQ.1 (SCV2_23C1D-04) and SARS-CoV-2 Omicron variant, sublineage BA.5 (SCV2_23C1D-05).

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for Influenza A was evaluated against the RNA from the following strains, showing positive results:

Influenza A Virus, A/Brisbane/59/2007 (H1N1) (FR-1), Influenza A Virus, A/South Dakota/6/2007 (H1N1) (FR-3), Influenza A Virus, A/Hawaii/31/2007 (H1N1) (FR-5), Influenza A Virus, A/Qatar/1123/2007 (H1N1) (FR-

6), Influenza A Virus, A/Cambodia/0371/2007 (H1N1) (FR-7), Influenza A Virus, A/Brisbane/10/2007 (H3N2) (FR-8), Influenza A Virus, A/Taiwan/760/2007 (H3N2) (FR-12), Influenza A Virus, A/Texas/71/2007 (H3N2) (FR-13), Influenza A Virus, A/Brisbane/10/2007 IVR-147 (H3N2) (FR-27), Influenza A Virus, A/Brisbane/59/2007 IVR-148 (H1N1) (FR-28), Influenza A Virus, A/South Dakota/6/2007 X-173 (H1N1) (FR-29), Influenza A Virus, A/California/07/2009 (H1N1)pdm09 (FR-201), Influenza A Virus, A/California/08/2009 (H1N1)pdm09 (FR-202), Influenza A Virus, A/New York/18/2009 (H1N1)pdm09 (FR-203), Influenza A Virus, A/Mexico/4108/2009 (H1N1)pdm09 (FR-245), Influenza A Virus, A/California/07/2009 NYMC X-179A (H1N1)pdm09 (FR-246), Influenza A/Victoria/2570/2019 (H1N1) and/or Influenza A/Darwin/6/2021 (H3N2) (Vaccine Fluarix Tetra 2022/2023), Influenza A/Victoria/4897/2022 (H1N1) and/or Influenza A/Darwin/6/2021 (H3N2) (Vaccine Fluarix Tetra 2023/2024), AMPLIRUN TOTAL SARS-CoV-2-FluA-FluB-RSV CONTROL (Vircell MBTC031-R) and Influenza A Virus, H3/H2N2 (CAP ID3-09 2023).

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for Influenza B was evaluated against the RNA from the following strains, showing positive results:

Influenza B Virus, B/Pennsylvania/7/2007 (Yamagata Lineage) (FR-16), Influenza B Virus, B/Santiago/4364/2007 (Yamagata Lineage) (FR-17), Influenza B Virus, B/Brisbane/3/2007 (Yamagata Lineage) (FR-18), Influenza B Virus, B/Pennsylvania/5/2007 (Victoria Lineage) (FR-19), Influenza B Virus, B/Victoria/304/2006 (Victoria Lineage) (FR-20), Influenza B Virus, B/Bangladesh/3333/2007 (Yamagata Lineage) (FR-183), AMPLIRUN TOTAL SARS-CoV-2-FluA-FluB-RSV CONTROL (MBTC031-R), Influenza B/Austria/1359417/2021 and/or Influenza B/Phuket/3073/2013 (Vaccine Fluarix Tetra 2022/2023), Influenza B/Austria/1359417/2021 and/or Influenza B/Phuket/3073/2013 (Vaccine Fluarix Tetra 2023/2024).

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for RSV was evaluated against the RNA from Human Respiratory Syncytial Virus A (Strain A-2) (FR-294), Human Respiratory Syncytial Virus, A 2000/3-4 (NR-28530), AMPLIRUN TOTAL SARS-CoV-2-FluA-FluB-RSV CONTROL (MBTC031-R), and Human Respiratory Syncytial Virus, B (INSTAND 359043) , showing positive results.

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for parainfluenza was evaluated against the RNA from Parainfluenza Virus Serotype 1 (NIBSC 08/176), Parainfluenza Virus Serotype 2 (NIBSC 08/178), Parainfluenza Virus Serotype 4 (NIBSC 08/180), Parainfluenza Virus 2 (PINFRNA101S-06) and Parainfluenza Virus 3 (PINFRNA22S-02), showing positive results.

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for coronavirus was evaluated against the RNA from Quantitative Synthetic Human coronavirus Strain NL63 RNA (VR-3263SD) and Coronavirus HKU (CVRNA22S-04) , showing positive results.

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for metapneumovirus was evaluated against the RNA from AMPLIRUN® METAPNEUMOVIRUS RNA CONTROL (MBC144-R), Human metapneumovirus (NIBSC 08/320) and Quantitative Synthetic Human metapneumovirus hMPV RNA (VR-3250SD), showing positive results.

The analytical reactivity of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System for adenovirus was evaluated against the DNA from the following strains, showing positive results:

Adenovirus Type 2, species C (0810110CF), Adenovirus Type 3, species B (0810062CFHI), Adenovirus Type 4, species E (0810070CFHI), Adenovirus Type 5, species C (0810020CF), Adenovirus Type 6 (Species C), strain Tonsil 99 (VR-6), Adenovirus Type 7A, species B (0810021CFHI), Adenovirus Type 15 (Species D), strain 35 [955, CH.38] (VR-16), Adenovirus Type 31 (Species A) Strain 1315/63 (VR-3343), Adenovirus Type 37 (0810119CFHI), Adenovirus Type 40, strain Dugan (0810084CFHI), Adenovirus Type 41 (Species F), strain Tak (0810085CFHI) and First WHO International Standard for Human Adenovirus DNA (NIBSC code: 16/324).

12.8. Metrological traceability

This assay is not designed for measuring purposes.

13. Clinical performance characteristics

The clinical performance of VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System was tested using nasopharyngeal swab collected by nursing staff using a flexible nylon sterile swab and placed into the sterile tube containing 3 ml of Universal Transport Media® (UTM®) (Copan). The results were as follows:

	Site	Sample type	Workflow	Target
1	Hospital Universitario Miguel Servet (Zaragoza, Spain)	Nasopharyngeal swabs (Retrospective study)	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	SARS-CoV-2
				Influenza A
				Influenza B
				RSV (types A and B)
				Parainfluenza (types 1, 2, 3 and 4)
				Coronavirus (OC43, NL63, 229 and HKU1)
				Metapneumovirus
				Adenovirus

Table 47. Site, sample type, workflow, and target.

True positive and negative values, false positive and negative values, sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and the likelihood ratios (LR) for VIASURE *Respiratory Virus Extended Mix* 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	PPV	NPV	LR+	LR-
1	Cobas® SARS-CoV-2 & Influenza A/B assay (Roche)	SARS-CoV-2	110	733	7	6	0.95 (0.89-0.98)	0.99 (0.98-0.99)	0.94 (0.88-0.97)	0.99 (0.98-0.99)	100.3 (47.9-209.8)	0.052 (0.02-0.11)
		Flu A	143	699	4	10	0.94 (0.88-0.97)	0.99 (0.98-0.99)	0.97 (0.93-0.99)	0.99 (0.97-0.99)	164.3 (61.8-436.8)	0.066 (0.04-0.12)
		Flu B	29	826	0	1	0.97 (0.83-0.99)	1 (0.99-1)	1 (0.88-1)	0.99 (0.99-1)	1574 (98.4-25172)	0.048 (0.01-0.23)
	Allplex™ RV Essential Assay (Seegene)	Influenza A	143	699	4	10	0.94 (0.88-0.97)	0.99 (0.98-0.99)	0.97 (0.93-0.99)	0.99 (0.97-0.99)	164.3 (61.8-436.8)	0.066 (0.04-0.12)
		Influenza B	29	826	0	1	0.97 (0.83-0.99)	1 (0.99-1)	1 (0.88-1)	0.99 (0.99-1)	1574 (98.4-25172)	0.048 (0.01-0.23)
		RSV (types A and B)	60	787	6	3	0.95 (0.87-0.99)	0.99 (0.98-0.99)	0.91 (0.82-0.96)	0.99 (0.98-0.99)	125.9 (56.61-279.9)	0.048 (0.01-0.15)
		Parainfluenza (types 1, 2, 3 and 4)	74	765	9	8	0.90 (0.82-0.96)	0.99 (0.98-0.99)	0.90 (0.82-0.95)	0.99 (0.98-0.99)	77.61 (40.38-149.2)	0.099 (0.05-0.19)
		Metapneumovirus	73	778	1	4	0.95 (0.87-0.99)	0.99 (0.99-1)	0.99 (0.93-0.99)	0.99 (0.98-0.99)	738.5 (104.1-5240)	0.052 (0.02-0.14)
		Adenovirus	64	786	5	1	0.99 (0.92-1)	0.99 (0.98-0.99)	0.93 (0.84-0.97)	0.99 (0.99-1)	155.8 (65-373.4)	0.015 (0.002-0.11)
	Allplex™ Respiratory Panel 3 (Seegene) + sequencing	Coronavirus (OC43, NL63, 229 and HKU1)	39	813	2	2	0.95 (0.84-0.99)	0.99 (0.99-1)	0.95 (0.84-0.99)	0.99 (0.99-1)	387.6 (96.9-1549.9)	0.049 (0.013-0.189)

Table 48. True positive (TP) and negative values (TN), false positive (FP) and false negative (FN) values, sensitivity, specificity, Positive Predictive Values (PPV), Negative Predictive Values (NPV) and the likelihood ratios (LR) for VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System.

In conclusion, results show high agreement to detect SARS-CoV-2, Influenza A, Influenza B, RSV (types A and B), parainfluenza (types 1, 2, 3 and 4), coronavirus (OC43, NL63, 229E and HKU1), metapneumovirus and adenovirus using VIASURE *Respiratory Virus Extended Mix* Real Time PCR Detection Kit for BD MAX™ System.

Bibliography

Abu-Raya, B., Viñeta Paramo, M., Reicherz, F., & Lavoie, P. M. (2023). Why has the epidemiology of RSV changed during the COVID-19 pandemic? *EClinicalMedicine*, *61*, 102089. https://doi.org/10.1016/j.eclinm.2023.102089

Bergeron, H. C., & Tripp, R. A. (2021). Immunopathology of RSV: An Updated Review. *Viruses*, *13*, 2478. https://doi.org/10.3390/v13122478

Buckwalter, S. P., Teo, R., Espy, M. J., Sloan, L. M., Smith, T. F., & Pritt, B. S. (2012). Real-time qualitative PCR for 57 human adenovirus types from multiple specimen sources. *Journal of Clinical Microbiology*, *50*(3), 766–771. https://doi.org/10.1128/JCM.05629-11

CDC |. (2023). *COVID-19*. https://www.cdc.gov/coronavirus/2019-ncov/your-health/about-covid-19.html

Datta, N. (2023). A review of molecular biology detection methods for human adenovirus. *AIMS Biophysics*, *10*(1), 95–120. https://doi.org/10.3934/BIOPHY.2023008

Fernández-Pérez, G. C., Oñate Miranda, M., Fernández-Rodríguez, P., Velasco Casares, M., Corral de la Calle, M., Franco López, Díez Blanco, M., & Cuchat, J. M. O. (2021). SARS-CoV-2: cómo es, cómo actúa y cómo se expresa en la imagen. *Radiologia*, *63*, 115–126. https://doi.org/10.1016/j.rx.2020.10.006

Friedman, N., Alter, H., Hindiyeh, M., Mendelson, E., Avni, Y. S., & Mandelboim, M. (2018). Human Coronavirus Infections in Israel: Epidemiology, Clinical Symptoms and Summer Seasonality of HCoV-HKU1. *Viruses*, *10*. https://doi.org/10.3390/v10100515

Gaunt, E. R., Hardie, A., Claas, E. C. J., Simmonds, P., & Templeton, K. E. (2010). Epidemiology and Clinical Presentations of the Four Human Coronaviruses 229E, HKU1, NL63, and OC43 Detected over 3 Years Using a Novel Multiplex Real-Time PCR Method. *JOURNAL OF CLINICAL MICROBIOLOGY*, *48*(8), 2940–2947. https://doi.org/10.1128/JCM.00636-10

Henrickson, K. J. (2003). Parainfluenza Viruses. *CLINICAL MICROBIOLOGY REVIEWS*, *16*(2), 242–264. https://doi.org/10.1128/CMR.16.2.242-264.2003

Hu, B., Guo, H., Zhou, P., & Shi, Z. L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology*, *19*, 141–154. https://doi.org/10.1038/s41579-020-00459-7

Ison, M. G., & Hayden, R. T. (2016). Adenovirus. *Microbiology Spectrum*, *4*(4), DMIH2-0020–2015. https://doi.org/10.1128/microbiolspec

Jansen, R. R., Schinkel, J., Koekkoek, S., Pajkrt, D., Beld, M., De Jong, M. D., & Molenkamp, R. (2011). Development and evaluation of a four-tube real time multiplex PCR assay covering fourteen respiratory viruses, and comparison to its corresponding single target counterparts. *Journal of Clinical Virology*, *51*, 179–185. https://doi.org/10.1016/j.jcv.2011.04.010

Krammer, F., Smith, G. J. D., Fouchier, R. A. M., Peiris, M., Kedzierska, K., Doherty, P. C., Palese, P., Shaw, M. L.,

Treanor, J., Webster, R. G., & García-Sastre, A. (2018). Influenza. *Nature Reviews Disease Primers*, *4*(3), 1–21. https://doi.org/10.1038/s41572-018-0002-y

Lim, Y. X., Ng, Y. L., Tam, J. P., & Liu, D. X. (2016). Human Coronaviruses: A Review of Virus-Host Interactions. *Diseases*, *4*(26), 1–28. https://doi.org/10.3390/diseases4030026

Lynch, J. P., & Kajon, A. E. (2016). Adenovirus: Epidemiology, Global Spread of Novel Serotypes, and Advances in Treatment and Prevention. *Seminars in Respiratory and Critical Care Medicine*, *37*, 586–602. https://doi.org/10.1055/s-0036-1584923

Safiabadi Tali, S. H., LeBlance, J. J., Sadiq, Z., Oyewunmi, O. D., Camargo, C., Nikpuor, B., Armanfard, N., Sagan, S. M., & Jahanshahi-Anbuhi, S. (2021). Tools and Techniques for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)/COVID-19 Detection. *Clinical Microbiology Reviews*, *34*(3), e00228-20.

Schuster, J. E., & Williams, J. V. (2013). Human Metapneumovirus. *Pediatrics in Review*, *34*(12), 558–565.

Templeton, K. E., Scheltinga, S. A., Beersma, M. F. C., Kroes, A. C. M., & Claas, E. C. J. (2004). Rapid and Sensitive Method Using Multiplex Real-Time PCR for Diagnosis of Infections by Influenza A and Influenza B Viruses, Respiratory Syncytial Virus, and Parainfluenza Viruses 1, 2, 3, and 4. *JOURNAL OF CLINICAL MICROBIOLOGY*, *42*(4), 1564–1569. https://doi.org/10.1128/JCM.42.4.1564-1569.2004

Tyrrell, carina S., Allen, J. L. Y., & Gkrania-Klotsas, E. (2021). Influenza: epidemiology and hospital management. *Medicine (Abingdon, England : UK Ed.)*, *49*(12), 797–804.

Uddin, S., & Thomas, M. (2020). Human Metapneumovirus. In *StatsPearls*. StatPearls Publishing. https://doi.org/10.1016/B978-0-323-40181-4.00226-7

Uyeki, T. M., Hui, D. S., Zambon, M., Wentworth, D. E., & Monto, A. S. (2022). Influenza. *The Lancet*, *400*, 693–706. https://doi.org/10.1016/S0140-6736(22)00982-5

WHO | World Health Organization. (n.d.). *Respiratory Syncytial Virus (RSV) disease*. Retrieved August 10, 2023, from https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/respiratory-syncytial-virus-disease

WHO | World Health Organization. (2023a). *Coronavirus disease (COVID-19)*. https://www.who.int/news-room/fact-sheets/detail/coronavirus-disease-(covid-19)

WHO | World Health Organization. (2023b). *Influenza (Seasonal)*. https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)

Zeng, Z.-Q., Chen, D.-H., Tan, W.-P., Qiu, S.-Y., Xu, D., Liang, H.-X., Chen, M.-X., Li, X., Lin, Z.-S., Liu, W.-K., & Zhou, R. (2018). Epidemiology and clinical characteristics of human coronaviruses OC43, 229E, NL63, and HKU1: a study of hospitalized children with acute respiratory tract infection in Guangzhou, China. *European Journal of Clinical Microbiology and Infectious Diseases*, *37*, 363–369. https://doi.org/10.1007/s10096-017-3144-z

Symbols for IVD components and reagents

IVD	*In vitro* diagnostic device		Keep dry		Use by		Manufacturer	LOT	Batch code
	Consult instructions for use		Temperature limitation	Σ	Contains sufficient for <n> test	UDI	Unique Device Identification	REF	Catalogue number
CE	CE marking		Keep away from sunlight						

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Change Control		
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00	Original version	01/08/2025

Table A 2. Control change table.

Revision: 1st August 2025

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