VIASURE

Real Time PCR Detection Kits

by CerTest BIOTEC

Flu A, Flu B & RSV

Handbook for the following references/

Priručnik za sljedeće reference:

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection Kit **BD REF 444200**

to be used with BD MAX™

koristi se sa BD MAX™

ENGLISH

1. Intended use

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit is designed for the specific identification and differentiation of Influenza A, Influenza B (Flu A and/or B) and/or Human Respiratory Syncytial Virus (RSV) in respiratory samples from patients with signs and symptoms of respiratory infection. This test is intended to be used as an aid in the diagnosis of Flu A, Flu B and/or RSV in combination with clinical and epidemiological risk factors. The assay uses the BD MAX™ System for extraction of RNA and subsequent Real Time RT-PCR employing the reagents provided combined with universal reagents and disposables for the BD MAX™ system. RNA from clinical specimens is detected using fluorescent reporter dye probes specific for Flu A, Flu B and RSV.

2. Summary and Explanation

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

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

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

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

3. Principle of the procedure

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit is designed for the diagnosis of Influenza A, Influenza B and/or RSV in respiratory samples. The detection is done in a one-step real time RT format where the reverse transcription and the subsequent amplification of the specific targeted sequence occur in the same reaction tube. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase which is followed by amplification of a conserved region of the *M1* gene for Flu A and Flu B and a conserved region of the *N* gene for RSV using specifics primers and a fluorescent-labelled probes.

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit 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 the target template. This fluorescence can be measured on BD MAX™ System.

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit contains in each tube all the components necessary for real time PCR assay (specific primers/probes, dNTPS, buffer, polymerase, reverse-transcriptase) in an stabilized format, as well as an internal control to monitor PCR inhibition. Influenza A RNA targets are amplified and detected in channel 475/520, Influenza B RNA in channel 585/630, RSV RNA in channel 630/665 and the internal control (IC) in channel 530/565.

4. Reagents provided

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit includes the following materials and reagents detailed in Table 1:

Reference	Reagent/Material	Description	Color	Amount
VS-ABR212R	*Flu A, Flu B & RSV* reaction tube	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers and Internal control in stabilized format	Transparent Red foil	2 pouches of 12 tubes
VS-RB05	Rehydration Buffer tube	Solution to reconstitute the stabilized product	Transparent Purple foil	1 pouch of 24 tubes

Table 1. Reagents and materials provided in VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit with Ref. VS-ABR124.

5. Reagents and equipment to be supplied by the user

The following list includes the materials and equipment that are required for use but not included in the VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit.

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

6. Transport and storage conditions

- The kits can be shipped and stored at 2-40ºC until the expiration date which is stated on the label.
- Keep components away from sunlight.

7. Precautions for users

- For professional in vitro diagnostic use.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective box is open or broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Protect reagents against from humidity. Prolonged exposure to humidity may affect product performance.
- 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 Flu A, Flu B & RSV Real Time PCR Detection kit, BD MAX™ ExK™ TNA-3 extraction kit, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Gloves must be changed before manipulating reagents and cartridges.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles and mask. Do not eat, drink or smoke in the working area. Once you finish the test wash your hands.
- Specimens must be treated as potentially infectious as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national safety regulations. Take necessary precautions during the collection, storage, treatment and disposal of samples.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Test procedure

8.1. SAMPLE COLLECTION, STORAGE AND TRANSPORT

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit has been validated on throat swabs that were obtained by flexible nasopharyngeal nylon flocked swabs, immediately placed in viral transport medium (Vircell, Spain). Additional respiratory specimens from symptomatic patients could be tested according to the literature (i.e. nasal/deep nasal/nasopharyngeal swabs, combined nasal and throat swab, nasopharyngeal/nasal/tracheal

aspirates, nasopharyngeal/nasal/throat washes, bronchoalveolare lavage (BALs), sputum), but must be validated by the user.

Collection, storage and transport specimens should be maintained per the conditions validated by the user. Overall, respiratory samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type), and processed as soon as possible to guarantee the quality of the test. The specimens must be transported following the local and national regulations for the transport of pathogen material. For long term transport (more than 24 hours), we recommend shipping at ≤-20°C. The samples can be stored at 2 to 8°C for up to 24 hours or frozen at -20°C or -70°C for conservation. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

8.2. SAMPLE PREPARATION AND RNA EXTRACTION

Perform the sample preparation according to the recommendations appearing in the instructions for use of extraction kit used, BD MAX™ ExK™ TNA-3. Note that some other specimens may require pre-processing. Application-specific extraction preparation procedures should be developed and validated by the user.

1. Pipette 200-400 µL of respiratory clinical specimen into a BD MAX™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute.

2. Proceed to BD MAX™ System Operation.

8.3. PCR PROTOCOL

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

8.3.1. Creating PCR test programme for VIASURE Flu A, Flu B & RSV Real Time PCR Detection kit

Note: If you have already created the VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection test, you can skip step 8.3.1 and go directly to 8.3.2.

1) On the "Run" screen of the BD MAX™ System, select the "Test Editor" tab.
2) Click the "Create" button.
3) In the "Test Name" window, name your test: i.e. VIASURE *Flu A, Flu B & RSV*.
4) In the "Extraction Type" drop down menu, select "ExK TNA-3".
5) In the "Master Mix Format" drop down menu, choose "Type 5"
    a. Note: Product may be used in combination with an additional Viasure for BD MAX test, then select "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)".
6) In the "Sample extraction parameters" select "User defined" and adjust sample volume to the volume of clinical specimen used plus 550 µL.
    a. Example: If pipette 200 µL of respiratory clinical specimen into a BD MAX TNA-3 Sample Buffer Tube then set parameter to 750 µL.
    b. Note: maximum setting is 950 µL.
7) In the "Ct Calculation" select "Call Ct at Threshold Crossing".

8) In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 2).

   a. Note: Product may be used in combination with an additional Viasure for BD MAX test, PCR Settings and Test Steps should be completed for snap 2 (green) and snap 4 (blue) positions.

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

Table 2. PCR settings.

9) In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 3), as well

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

Table 3. Spectral cross-talk parameters.

10) In "Test Steps" tab, enter the PCR protocol (Table 4).

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

Table 4. PCR protocol.

11) Click the "Save Test" button.

8.3.2. BD MAX™ Rack set up

1) For each specimen to be tested, remove one Unitized Reagent Strips (BD MAX™ TNA Reagent Strip (TNA)) 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 (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 VIASURE *Flu A, Flu B & RSV* reaction tubes (red foil) and snap into their corresponding positions in the strip (Snap position 2, green color coding on the rack. See Figure
   a. Remove excess air, and close aluminum pouches with the zip seal.
   b. In order to carry on 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.

      Note: If you choose the format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Section 8.3.1), determine and separate the appropriate number

of additional VIASURE reaction tubes (different foil) and snap into their corresponding positions in the strip (Snap position 4, blue color coding on the rack. See Figure 1). Remove excess air, and close aluminum pouches with the zip seal.

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

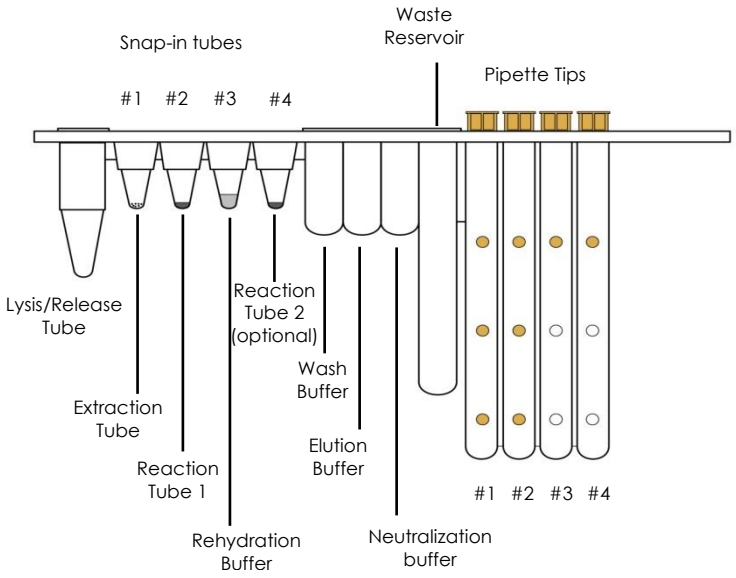


Figure 1. BD MAX™ TNA Reagent Strip (TNA) from the BD MAX™ ExK TNA-3 kit.

8.3.3. BD MAX™ Instrument set up

1) Select the "Work List" tab on the "Run" screen of the BD MAX™ System software v4.50A or higher.
2) In the "Test" drop down menu, select VIASURE *Flu A, Flu B & RSV* (if not already created see Section 8.3.1).
3) Select the appropriate kit lot number (found on the outer box of extraction kit used) from the pull down menu (optional).
4) Enter the Sample Buffer Tube identification number into the Sample tube window of the Worklist, either by scanning the barcode with the scanner or by manual entry.
5) Fill the Specimen/Patient ID into Accession window of the Worklist (if applicable) and click the "Save" button. Continue until all Sample Buffer tubes are entered. Ensure that the specimen/patient ID and the Sample Buffer tubes are accurately matched.
6) Place the prepared Sample Buffer Tube into the BD MAX™ Rack(s).
7) Load the rack(s) into the BD MAX™ System (Rack A is positioned on the left side of the BD MAX™ System and Rack B on the right side).
8) Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System.
9) Close the BD MAX™ System door.
10) Click "Start Run" to begin the procedure.

8.3.4 BD MAX™ Report

1) In main menu, click the "Results" button.

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

9. Result interpretation

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

The analysis of the data is done by the BD MAX™ software according to manufacturer´s instructions. The BD MAX™ software reports Ct values and amplification curves for each detector channel of each specimen 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 4). Amplification curve of the sample showing a "0" Ct value must be checked manually.

- Ct value of -1 indicates that no amplification process has occurred.

- Any other Ct value should be interpreted in correlation with the amplification curve and according to the sample interpretation guidelines outlined in Table 5.

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

Using the following table read and analyze the results:

Flu A (475/520)	Flu B (585/630)	RSV (630/665)	Internal control (530/565)	Interpretation
-	-	-	+	**Flu A, Flu B and RSV Negative**
+	+	+	+/-	**Flu A, Flu B and RSV Positive**
+	-	-	+/-	**Flu A Positive, Flu B and RSV Negative**
+	+	-	+/-	**Flu A and Flu B Positive, and RSV Negative**
+	-	+	+/-	**Flu A and RSV Positive, and Flu B Negative**
-	+	-	+/-	**Flu B Positive, Flu A and RSV Negative**
-	+	+	+/-	**Flu B and RSV Positive, Flu A Negative**
-	-	+	+/-	**RSV Positive, Flu A and Flu B Negative**
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.**
IND	IND	IND	IND	**Indeterminate assay result (IND). Due to BD MAX™ System failure. Assay result displayed in case of an instrument failure linked to an error code.**
INC	INC	INC	INC	**Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run.**

Table 5. Sample interpretation
+: Amplification occured
-: No amplification occured

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

A sample is considered negative, if the sample shows no amplification signal in the detection system but the internal control is positive. An inhibition of the PCR reaction can be excluded by the amplification of internal control.

In case of unresolved results, absence of internal control signal in negative sample we recommend to repeat the assay diluting the sample 1:10 to check for possible problems of inhibition.

The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

10. Limitations of the test

- The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.
- Although this assay can be used with other types of samples it has been validated with throat swabs.
- The quality of the test depends on the quality of the sample; proper RNA from clinical samples must be extracted. Unsuitable collection, storage and/or transport of specimens may give false negative results.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- There is a possibility of false positive results due to cross-contamination by Flu A, Flu B and/or RSV, either samples containing high concentrations of target RNA or contamination due to PCR products from previous reactions.
- The results obtained with VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection Kit may be Unresolved due to the sample contains inhibitors or incorrect rehydration of the lyophilized reaction mix tube, or be Indeterminate or Incomplete due to instrument failure, and require retesting.

11. Quality control

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection Kit contains an internal control (IC) in each reaction tube which confirms the correct performance of the technique.

12. Performance characteristics

12.1. Clinical sensitivity and specificity

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

The results were as follows:

VIASURE *Flu A, B & RSV* Real Time PCR Detection kit	cobas® Influenza A/B & RSV (Roche)			
		+	-	Total
	+	157	2*	159
	-	7*	178	185
	Total	164	180	344

Table 6. Comparative results for Flu A.

Positive percent agreement is >98% and negative percent agreement is >96%.

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

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit	cobas® Influenza A/B & RSV (Roche)			
		+	-	Total
	+	99	4*	103
	-	1*	240	241
	Total	100	244	344

Table 7. Comparative results for Flu B.

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

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

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit	cobas® Influenza A/B & RSV (Roche)			
		+	-	Total
	+	22	4*	26
	-	3*	315	318
	Total	25	319	344

Table 8. Comparative results for RSV.

Positive percent agreement is >84% and negative percent agreement is >99%.

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

The results show a high sensitivity and specificity to detect Influenza A, Influenza B and/or RSV viruses using VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit.

12.2. Analytical sensitivity

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit has a detection limit of ≥10 RNA copies per reaction for Flu A, Flu B and RSV with a positive rate of ≥95% (Figure 2, 3 and 4).

Figure 2. Dilution series of Flu A (2*10⁶-2*10¹ copies/rxn) template run on the BD MAX™ System (475/520 (FAM) channel).

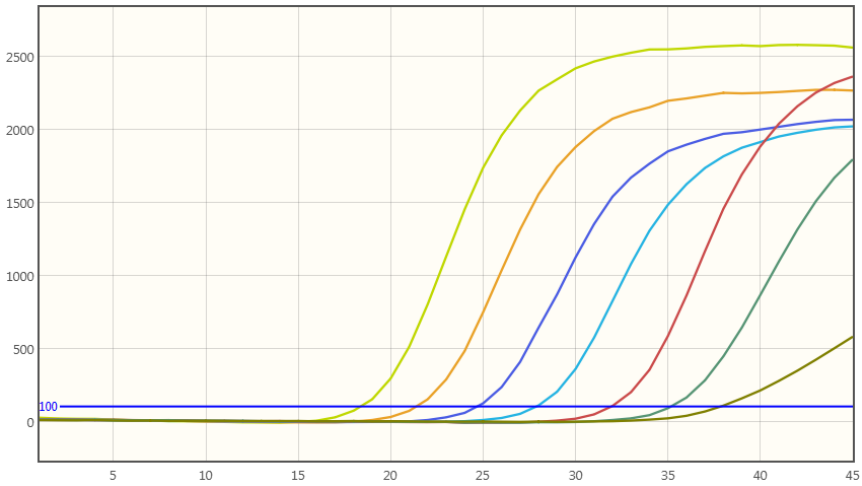


Figure 3. Dilution series of Flu B (2*10⁶-2*10¹ copies/rxn) template run on the BD MAX™ System (585/630 (ROX) channel).

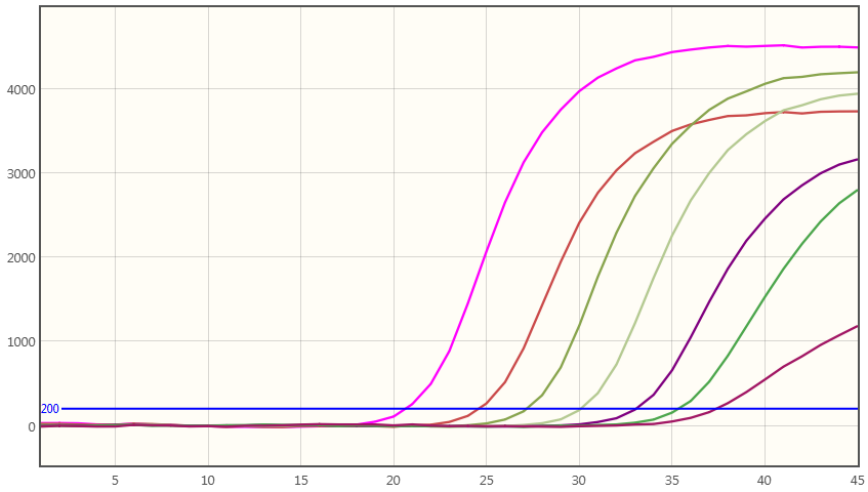
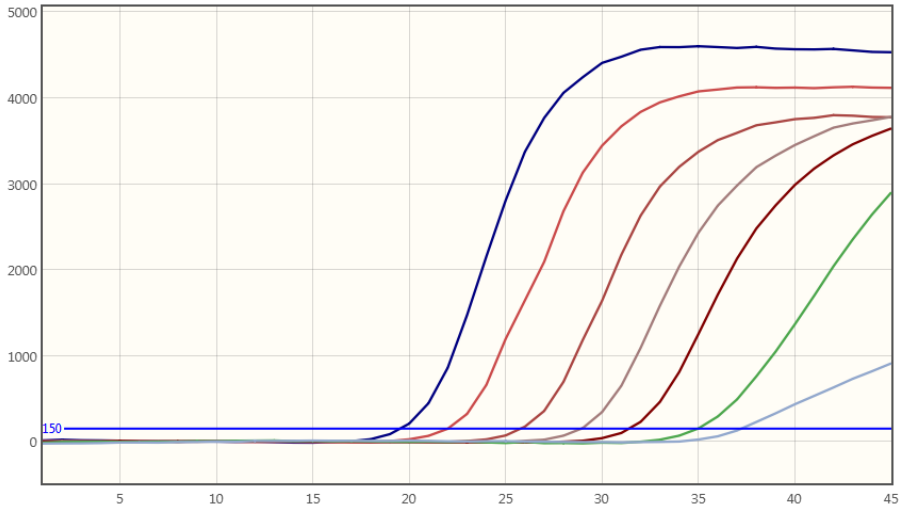


Figure 4. Dilution series of RSV (2*10⁶-2*10⁰ copies/rxn) template run on the BD MAX™ System (630/665 (Cy5) channel).



12.3. Analytical specificity

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

Cross-reactivity testing					
Bordetella pertussis	-	Human rhinovirus	-	A/Mallard/Netherlands/2/2009 (H7N7) virus	-/+
Bordetella parapertussis	-	Human Adenovirus	-	Influenza A/Anhui/1/2013 (H7N9) virus	-/+
Legionella pneumophila	-	MERS Coronavirus	-	Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2)	-/+
Mycoplasma pneumoniae	-	Influenza A/New Caledonia/20/99(H1N1) virus	-/+	Influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2)	-/+
Streptococcus pneumoniae	-	Influenza A/California/7/2009(H1N1)pdm09-like virus	-/+	Influenza A/mallard/Netherlands/12/2000 (H7N7) - IBCDC-1	-/+
Staphylococcus aureus subsp. *aureus*	-	Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-/+	Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4	-/+
Methicillin-resistant *Staphylococcus aureus*	-	Influenza A/Perth/16/2009(H3N2)-like virus	-/+	Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2)	-/+
Haemophilus influenzae MinnA	-	Influenza A/Thüringen/5/17 (H3N2) virus	-/+	Influenza A/South Australia/55/2014	-/+
Moraxella catarrhalis	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-/+	Influenza A/Uruguay/716/2007 (H3N2)(NYMC-175C)	-/+
Chlamydia psittaci genotype A and C	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-/+	Influenza B/Brisbane/60/2008-like virus	-/+
Chlamydophila pneumoniae	-	Influenza A/Turkey/Germany R2485+86/2014 (H5N8) virus	-/+	Influenza B/Florida/04/06 virus	-/+
Enterovirus 68 and 71	-	Influenza A/DE-SH/Reiherente/AR8444/ 2013 (H5N8) virus	-/+	Influenza B/Phuket/3073/2013 virus	-/+
Enterovirus Echovirus types 11 and 30	-	A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1)	-/+	Influenza B/Colorado/6/2017	-/+
Human parainfluenza 1, 2, 3 and 4 viruses	-	A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a)	-/+	Influenza B/Maryland/15/2016	-/+
Human metapneumovirus A and B	-	A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a)	-/+	Respiratory syncytial virus (RSV)	-/+
Human coronavirus 229E	-	A/Hong Kong/213/2003 (H5N1) virus	-/+		

Table 9. Reference pathogenic microorganisms used in this study.

12.4. Analytical reactivity

The reactivity of VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit for Flu A was evaluated against strains: A/New Caledonia/20/99(H1N1) virus, A/California/7/2009(H1N1)pdm09-like virus, A/Michigan/45/2015 (H1N1)pdm09 virus, A/Perth/16/2009(H3N2)-like virus, A/Thüringen/5/17 (H3N2) virus, A/Switzerland/9715293/2013 (H3N2) virus, A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus, A/Turkey/Germany R2485+86/2014 (H5N8) virus, A/DE-SH/Reiherente/AR8444/ 2013 (H5N8) virus, A/Anhui/1/2013 (H7N9) virus, A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2), A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2), A/mallard/Netherlands/12/2000 (H7N7) - IBCDC-1, A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4, A/Singapore/INFIMH-16-0019/2016 (H3N2), A/South Australia/55/2014 and A/Uruguay/716/2007 (H3N2)(NYMC-175C), A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1) A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a), A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a), A/Hong Kong/213/2003 (H5N1) and A/Mallard/Netherlands/2/2009 (H7N7) virus, showing positive results.

The reactivity of VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit for Flu B was evaluated against strains: B/Brisbane/60/2008-like virus (B/Victoria lineage), B/Florida/04/06 and B/Phuket/3073/2013 (B/Yamagata lineage), B/Colorado/6/2017, B/Maryland/15/2016 showing positive results.

The reactivity of VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit for RSV was evaluated against Human Respiratory Syncytial Virus (RSV A and B), showing positive results.

HRVATSKI

1. Namjena

Komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit dizajniran je za specifičnu identifikaciju i diferencijaciju gripe tipa A, gripe tipa B i/ili humanog respiratornog sincicijskog virusa (RSV) u respiratornim uzorcima od bolesnika sa znakovima i simptomima respiratorne infekcije. Predviđeno je da se ovaj test koristi kao pomoć u dijagnosticiranju gripe tipa A, gripe tipa B i virusa RSV u kombinaciji s kliničkim i epidemiološkim faktorima rizika. Test koristi BD MAX™ sustav za ekstrakciju RNK, a zatim lančanu reakciju polimeraze reverznom transkripcijom (RT-PCR) u stvarnom vremenu s priloženim reagensima u kombinaciji s univerzalnim reagensima i jednokratnim materijalom za BD MAX™ sustav. Detekcija RNK iz kliničkih uzoraka vrši se primjenom sondi s fluorescentnim indikacijskim bojilom specifičnim za gripu tipa A, gripu tipa B i RSV.

2. Sažetak i objašnjenje

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

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

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

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

3. Načelo postupka

Komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit dizajniran je za dijagnosticiranje gripe tipa A, gripe tipa B i/ili virusa RSV u respiratornim uzorcima, Detekcija se obavlja u obliku reverzne transkripcije (RT) u stvarnom vremenu koja se sastoji od jednog koraka, gdje se reverzna transkripcija i potom amplifikacija specifične ciljane sekvence vrši u istoj reakcijskoj epruveti. Nakon izolacije RNK, vrši se njena transkripcija čime se dobiva komplementarna DNKA zahvaljujući reverznoj transkriptazi, nakon koje slijedi amplifikacija konzervirane regije gena *M1* za gripu tipa A i gripu tipa B te konzervirane regije gena *N* za virus RSV primjenom specifičnih početnica i fluorescentno obojanih sondi.

Komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit temelji se na djelovanju 5' egzonukleaze u DNK polimerazi. Tijekom amplifikacije DNK ovaj enzim rascjepljuje sondu vezanu za sekvencu komplementarne DNK, čime se razdvaja supresorsku boju od indikacijskog gena. Ova reakcija dovodi do povećanja fluorescencijskog signala srazmjernog količini ciljnog predloška. Ta fluorescencija može se izmjeriti pomoću BD MAX™ sustava.

Komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit sadrži u svakoj epruveti sve potrebne komponente za obavljanje testa PCR-a (specifične početnice/sponde, dNTPS pufer, polimerazu, reverznu transkriptazu) u stabiliziranom obliku, kao i unutarnju kontrolu za nadzor inhibicije PCR-a. RNK virusa gripe tipa A je pojačana i otkrivena u kanalu 475/520, RNK virusa gripe tipa B u kanalu 585/630, RNK virusa RSV u kanalu 630/665, a unutarnja kontrola (IC) u kanalu 530/565.

4. Reagensi koji se isporučuju

Komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit sadrži sljedeće materijale i reagense navedene u tablici 1:

Referenca	Reagens/materijal	Opis	Boja	Količina
VS-ABR212R	*Flu A, Flu B & RSV* reaction tube	Smjesa enzima, sondi/početnica, pufera, dNTP-ova, stabilizatora i unutarnje kontrole u stabiliziranom obliku	Prozirno Crvena folija	2 vrećice s 12 epruveta
VS-RB05	Rehydration Buffer tube	Otopina za rekonstituciju stabiliziranog proizvoda	Prozirno Ružičasta folija	1 vrećica s 24 epruvete

Tablica 1. Reagensi i materijali koji se nalaze u kompletu VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit s referentnim brojem VS-ABR124.

5. Reagensi i oprema koju mora pribaviti korisnik

Sljedeći popis uključuje materijale i opremu koja je neophodna za korištenje, ali nije dio kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit.

- Instrument za lančanu reakciju polimeraze (PCR) u stvarnom vremenu: BD MAX™ sustav.
- BD MAX™ ExK™ TNA-3 (Ref: 442828)
- BD MAX™ PCR Cartridges (Ref: 437519)

- Vortex.
- Mikropipete (precizne u rasponu od 2 do 1000 µl).
- Nastavci za filtar.
- Nenaprašene jednokratne rukavice.

6. Uvjeti za transport i pohranu

- Kompleti se mogu transportirati i čuvati na temperaturi od 2-40°C do isteka roka valjanosti navedenog na naljepnici.
- Čuvajte komponente dalje od sunčeve svjetlosti.

7. Mjere opreza za korisnike

- Samo za *in vitro* dijagnostičku upotrebu.
- Nemojte koristiti reagense i/ili materijale kojima je istekao rok valjanosti.
- Nemojte koristiti komplet ako je potrgana naljepnica kojom je zatvorena vanjska kutija.
- Nemojte koristiti reagense ako je zaštitna kutija otvorena ili oštećena po prispijeću.
- Nemojte koristiti reagense ako su zaštitne vrećice otvorene ili oštećene po prispijeću.
- Nemojte koristiti reagense ako desikant u vrećicama s reagensima nije prisutan ili je oštećen.
- Nemojte vaditi desikant iz vrećica s reagensima.
- Nakon svake upotrebe odmah zatvorite zaštitne vrećice s reagensima pomoću patentnog zatvarača. Prije zatvaranja istisnite višak zraka iz vrećica.
- Nemojte koristiti reagense ako je folija potrgana ili oštećena.
- Nemojte miješati reagense iz različitih vrećica, kompleta i/ili serija.
- Zaštitite reagense od vlage. Duže izlaganje vlazi može utjecati na učinak proizvoda.
- U slučajevima gdje se drugi testovi PCR-om obavljaju u istom općem prostoru laboratorija potreban je oprez kako ne bi došlo do kontaminacije kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit, kompleta BD MAX™ ExK™ TNA-3 extraction kit, svih dodatnih reagensa potrebnih za testiranje i BD MAX™ sustava. Rukavice se moraju promijeniti prije rukovanja reagensima i patronama.
- Koristite jednosmjeran radni proces. Treba započeti u području za ekstrakciju, a zatim preći u područje za amplifikaciju i detekciju. nemojte vraćati uzorke, opremu u reagense u područje u kojem je obavljen prethodni korak.
- Pridržavajte se dobre laboratorijske prakse. Nosite zaštitnu odjeću, jednokratne rukavice, zaštitne naočale i masku. Nemojte jesti, piti niti pušiti u radnom prostoru. Nakon što završite test, operite ruke.
- Uzorci se moraju smatrati potencijalno zaraznim, a isto vrijedi za reagense i materijale koji su bili izloženi uzorcima i mora se rukovati u skladu s nacionalnim sigurnosnim propisima. Poduzmite neophodne mjere opreza prilikom prikupljanja, pohrane, tretiranja i odlaganja uzoraka u otpad.
- Preporučuje se redovita dekontaminacija opreme koja se često koristi, naročito mikropipeta i radnih površina.
- Posavjetujte se s Korisničkim priručnikom za BD MAX™ sustav u vezi s dodatnim upozorenjima, mjerama opreza i postupcima.

8. Testni postupak

8.1. PRIKUPLJANJE, POHRANA I TRANSPORT UZORAKA

Komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit validiran je na brisevima iz grla dobivenim pomoću fleksibilnih nazofaringealnih štapića od najlona, nakon čega su uzorci odmah stavljeni u virusni transportni medij (Vircell, Španjolska). Dodatni respiratorni uzorci od simptomatski bolesnika mogu se testirati prema literaturi (tj. nazalni/duboki nazalni/nazofaringealni štapići, kombinirani nazalni štapići i štapići za grlo, nazofaringealni/nazalni/trahealni aspirati, uzorci dobiveni ispiranjem nazofarinksa/nosa/grla, brohnoalveolarni lavaž (ispljuvak), ali ih korisnik mora validirati.

Prikupljanje, pohrana i transport uzoraka treba obavljati u uvjetima koje je validirao korisnik. U globalu, respiratorne uzorke treba prikupiti i označiti pravilno u čiste spremnike sa ili bez transportnog medija (ovisno o vrsti uzorka) i obraditi što je moguće prije u cilju osiguranja kvalitete testa. Uzorci se moraju transportirati u skladu s lokalnim i nacionalnim propisima za transport patogenih materijala. Za dugotrajni transport (duži od 24 sata) preporučujemo otpremanje na temperaturi od ≤-20 °C. Uzorci se mogu čuvati na temperaturi od 2 do 8 °C tijekom 24 sata ili držati smrznuti na temperaturi od -20°C ili -70°C za pohranu. Treba izbjegavati ponavljanje ciklusa smrzavanja i odmrzavanja kako bi se spriječilo propadanje uzorka i nukleinskih kiselina.

8.2. PRIPREMA UZORAKA I EKSTRAKCIJA RNK

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

1. Pipetom prenesite 200-400 µl respiratornog kliničkog uzorka u BD MAX™ TNA-3 epruvetu za uzorak s puferom te je zatvorite čepom s membranom. Izmiješajte uzorak vrtloženjem pri visokoj brzini tijekom 1 minute.

2. Pređite na rad sa BD MAX™ sustavom.

8.3. PROTOKOL ZA PCR

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

8.3.1. Kreiranje programa za testiranje PCR-om za komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit

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

1) Na zaslonu „Run“ (Pokreni) na BD MAX™ sustavu odaberite karticu „Test Editor“ (Uređivač testa).
2) Kliknite na gumb „Create“ (Kreiraj).
3) U prozoru „Test Name“ (Naziv testa) dajte ime svom testu, npr. VIASURE *gripa A, gripa B i RSV*.
4) S padajućeg izbornika „Extraction Type“ (Vrsta ekstrakcije) odaberite „ExK TNA-3“.
5) S padajućeg izbornika „Master Mix Format“ (Oblik glavne smjese) odaberite „Type 5“ (Tip 5)

a. Napomena: Proizvod se može koristiti u kombinaciji s dodatnim kompletom Viasure za BD MAX test, a u tom slučaju odaberite opciju „Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)".

6) U „Sample extraction parameters" (Parametri ekstrakcije uzorka) odaberite „User defined" (Korisnički definirani) i prilagodite volumen uzorka volumenu korištenog kliničkog uzorka plus 550 µl.

a. Primjer: Ako ste pipetom prenijeli 200 µl respiratornog kliničkog uzorka u BD MAX TNA-3 epruvetu za uzorak s puferom, postavite parametar na 750 µl.

b. Napomena: maksimalno možete postaviti 950 µl.

7) U „Ct Calculation" (Ct izračun) odaberite „Call Ct at Threshold Crossing" (Izračunaj Ct pri prelasku granice).

8) Na kartici „PCR settings" (Postavke za PCR) unesite sljedeće parametre: „Channel Settings" (Postavke kanala), „Gains" (Dobit) i „Threshold" (Granica) (Tablica 2).

a. Napomena: Proizvod se može koristiti u kombinaciji s dodatnim kompletom Viasure za BD MAX test, a u tom slučaju „PCR Settings" (Postavke za PCR) i „Test Steps" (Koraci testa) treba popuniti za položaje 2 (zeleni) i 4 (plavi).

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

Tablica 2. Postavke za PCR.

9) Na kartici „PCR settings" (Postavke za PCR) unesite i sljedeće parametre: „Spectral Cross Talk" (Tablica 3)

		False Receiving Channel (Kanal s lažnim rezultatima)				
	Channel (Kanal)	475/520	530/565	585/630	630/665	680/715
Excitation Channel (Kanal za ekscitaciju)	475/520	-	0,0	0,0	0,0	0,0
	530/565	0,0	-	2,0	0,0	0,0
	585/630	0,0	0,0	-	0,0	0,0
	630/665	0,0	0,0	4,0	-	0,0
	680/715	0,0	0,0	0,0	0,0	-

Tablica 3. Parametri spektralnog preklapanja signala

10) Na kartici „Test Steps" (Koraci testa) unesite protokol za PCR (Tablica 4).

Step Name (Naziv koraka)	Profile Type (Vrsta profila)	Cycles (Ciklusi)	Time (s) (Vrijeme (s))	Temperature (Temperatura)	Detect (Detekcija)
Reverse transcription	Hold	1	900	45 °C	-
Initial denaturation	Hold	1	120	98 °C	-
Denaturation and Annealing/Extension (Data collection)	2-Temperature	45	10	95 °C	-
			61,1	63 °C	✓

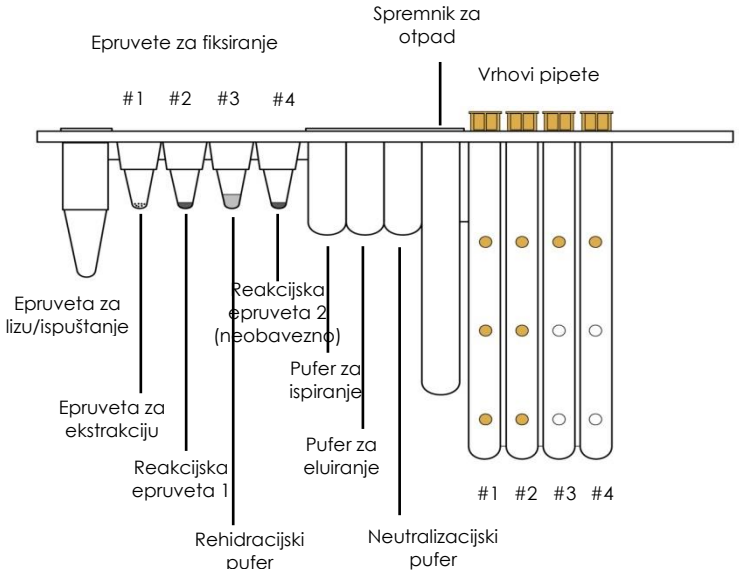
Tablica 4. Protokol za PCR.

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

8.3.2. Postavljanje BD MAX™ stalka

1) Za svaki uzorak koji treba testirati izvadite pojedinačne trake s reagensima u bloku (BD MAX™ TNA traka s reagensom (TNA)) iz kompleta BD MAX™ ExK TNA-3 kit. Lagano udarite svaku traku od čvrstu površinu kako biste osigurali da se sve tekućine nalaze na dnu epruveta i stavite ih na stalke za uzorke BD MAX™ sustava.
2) Izvadite potrebni broj BD MAX™ ExK™ TNA epruveta za ekstrakciju (B4) iz njihove zaštitne vrećice. Postavite epruvetu/e za ekstrakciju (bijela folija) u njihove odgovarajuće položaje u TNA (položaj 1 s oznakom u boji na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite vrećicu patentnom zatvaračem.
3) Odredite i razdvojite odgovarajući broj epruveta VIASURE *Flu A, Flu B & RSV* reaction tubes (crvena folija) i postavite ih u njihove odgovarajuće položaje na traci (položaj 2, označen zelenom bojom na stalku. Pogledajte Sliku
    a. Istisnite višak zraka i zatvorite aluminijske vrećice patentnim zatvaračem.
    b. U cilju obavljanja pravilnog postupka rehidracije pazite da se liofilizirani proizvod nalazi na dnu epruvete te da ne prianja za gornji dio epruvete ili za zatvarač od folije.

        Napomena: Ako odaberete oblik „Dual Master Mix Concentrated Lyophilized MM with rehydration Buffer (Type 5)“ (odjeljak 8.3.1), odredite i razdvojite odgovarajući broj dodatnih VIASURE reakcijskih epruveta (drugačija folija) i postavite ih u njihove odgovarajuće položaje na traci (položaj 4, označen plavom bojom na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite aluminijske vrećice patentnim zatvaračem.
4) Izvadite odgovarajući broj epruveta Rehydration Buffer tubes (ružičasta folija) i postavite ih u njihove odgovarajuće položaje na traci (položaj 3, nije obojen na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite vrećicu patentnim zatvaračem.
    a. U cilju obavljanja pravilnog prijenosa pazite da se tekućina nalazi na dnu epruvete te da ne prianja za gornji dio epruvete ili za zatvarač od folije.



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

8.3.3. Postavljanje BD MAX™ instrumenta

1) Odaberite karticu „Work List“ (Radni popis) na zaslonu „Run“ (Pokreni) na BD MAX™ sustavu s verzijom softvera v4.50A ili novijom.

2) S padajućeg izbornika „Test“ odaberite VIASURE *Flu A, Flu B & RSV* (ako test nije već kreairan, pogledajte odjeljak 8.3.1).
3) Odaberite odgovarajući broj serije kompleta (naveden na vanjskoj kutiji kompleta za ekstrakciju kojeg koristite) s padajućeg izbornika (neobavezno).
4) Unesite identifikacijski broj epruvete za uzorak s puferom u prozor „Sample tube“ (Epruveta s uzorkom) na kartici „Worklist“ (Radni popis) skeniranjem crtičnog koda pomoću sekenera ili ručnim unošenjem broja.
5) Popunite polje „Specimen/Patient ID“ (ID uzorka/bolesnika) u prozoru „Accession“ (Pristup) na kartici „Worklist“ (Radni popis) i kliknite na gumb „Save“ (Spremi). Nastavite s postupkom dok ne unesete sve epruvete za uzorak s puferom. Pazite da se ID uzorka/bolesnika i epruvete za uzorak s puferom podudaraju.
6) Stavite pripremljenu epruvetu za uzorak s puferom na BD MAX™ stalak/e.
7) Stavite stalak/e u BD MAX™ sustav (stalak A se nalazi lijevo u odnosu na BD MAX™ sustav, dok se stalak B nalazi na desnoj strani).
8) Stavite potrebni broj BD MAX™ PCR patrona u BD MAX™ sustav.
9) Zatvorite vrata BD MAX™ sustava.
10) Kliknite „Start Run“ (Pokreni postupak) da započnete postupak.

8.3.4 BD MAX™ izvješće

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

9. Tumačenje rezultata

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

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

- Ct vrijednost 0 znači da softver nije izračunao Ct vrijednost s navedenom granicom (pogledajte Tablicu 4). Amplifikacijska krivulja uzorka koja pokazuje „0“ za Ct vrijednost mora se provjeriti ručno.

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

- Svaku drugu Ct vrijednost treba tumačiti u vezi s amplifikacijskom krivuljom i prema smjernicama za tumačenje uzoraka navedenim u Tablici 5.

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

Pomoću sljedeće tablice očitajte i analizirajte rezultate:

Gripa tipa A (475/520)	Gripa tipa B (585/630)	RSV (630/665)	Unutarnja kontrola (530/565)	Tumačenje
-	-	-	+	**Negativno na gripu tipa A, gripu tipa B i virus RSV**
+	+	+	+/-	**Pozitivno na gripu tipa A, gripu tipa B i virus RSV**
+	-	-	+/-	**Pozitivno na gripu tipa A, negativno na gripu tipa B i virus RSV**
+	+	-	+/-	**Pozitivno na gripu tipa A i gripu tipa B, a negativno na virus RSV**
+	-	+	+/-	**Pozitivno na gripu tipa A i virus RSV, a negativno na gripu tipa B**
-	+	-	+/-	**Pozitivno na gripu tipa B, negativno na gripu tipa A i virus RSV**
-	+	+	+/-	**Pozitivno na gripu tipa B i virus RSV, negativno na gripu tipa A**
-	-	+	+/-	**Pozitivno na virus RSV, negativno na gripu tipa A i gripu tipa B**
UNR	UNR	UNR	UNR	**Neriješen (UNR) rezultat dobiven u prisutnosti inhibitora reakcije PCR-a ili kada dođe do nekog općeg problema (koji nije pokriven šifrom o pogrešci) pri obradi uzorka i/ili amplifikaciji.**
IND	IND	IND	IND	**Rezultat testa nije moguće utvrditi (IND). Zbog kvara BD MAX™ sustava. Rezultat testa prikazan u slučaju kvara instrumenta povezanog sa šifrom o pogrešci.**
INC	INC	INC	INC	**Nepotpun rezultat testa (INC). Zbog kvara BD MAX™ sustava. Rezultat testa prikazan u slučaju neuspješnog obavljanja postupka.**

Tablica 5. Tumačenje rezultata
+: Došlo je do amplifikacije
-: Nije došlo do amplifikacije

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

Uzorak se smatra negativnim ako ne pokazuje amplifikacijski signal u sustavu detekcije, ali je unutarnja kontrola pozitivna. Inhibiranje reakcije PCR-a može se isključiti amplifikacijom unutarnje kontrole.

U slučaju neriješenih rezultata, ako signal unutarnje kontrole nije prisutan u negativnom uzorku, preporučuje se ponavljanje testa razrjeđivanjem uzorka u omjeru 1:10 kako bi se provjerilo postojanje eventualnih problema s inhibicijom.

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

10. Ograničenja testa

- Rezultate testa treba procijeniti zdravstveni djelatnik uzimajući u obzir anamnezu, kliničke simptome i druge dijagnostičke testove.
- Iako se ovaj test može koristiti s drugim vrstama uzoraka, validiran je s brisovima grla.

- Kvaliteta testa ovisi o kvaliteti uzorka; mora se ekstrahirati odgovarajuća RNK iz kliničkih uzoraka. Nepravilno prikupljanje, pohrana i/ili transport uzoraka može dovesti do lažnih negativnih rezultata.
- Izuzetno niske razine ciljnog elementa ispod razine detekcije mogu biti detektirane, no moguće je da se rezultati neće moći ponoviti.
- Postoji mogućnost pojave lažnih pozitivnih rezultata zbog unakrsne kontaminacije izazvane gripom tipa A, gripom tipa b i/ili virusom RSV, bilo u slučaju da uzorci sadrže visoke koncentracije ciljne RNK ili kontaminacije ili u slučaju kontaminacije izazvane proizvodima koji sadrže PCR iz ranijih reakcija.
- Rezultati dobiveni primjenom kompletom VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit mogu biti neriješeni jer uzorak sadrži inhibitore ili zbog nepravilne rehidracije epruvete s liofiliziranom reakcijskom smjesom, dok zbog kvara instrumenta rezultati mogu biti neutvrdivi ili nepotpuni, u slučaju čega je potrebno ponovno testiranje.

11. Kontrola kvalitete

Komplet VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit sadrži unutarnju kontrolu u svakoj reakcijskoj epruveti pomoću koje se potvrđuje pravilno provođenje tehnike.

12. Radne karakteristike

12.1. Klinička osjetljivost i specifičnost

Klinički učinak kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit testiran je na 344 respiratorna uzorka (brisovi grla) od simptomatskih bolesnika. Ti su rezultati uspoređeni s rezultatima dobivenim pomoću metode molekularne detekcije (cobas® Influenza A/B & RSV (Roche)).

Dobiveni su sljedeći rezultati:

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit	**cobas® Influenza A/B & RSV (Roche)**			
		+	**-**	**Ukupno**
	+	157	2*	159
	-	7*	178	185
	Ukupno	164	180	344

Tablica 6. Komparativni rezultati za gripu tipa A.

Postotak podudarnosti pozitivnih rezultata je >98 %, a postotak podudarnosti negativnih rezultata je >96 %.

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

	cobas® Influenza A/B & RSV (Roche)			
		+	-	Ukupno
VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit	+	99	4*	103
	-	1*	240	241
	Ukupno	100	244	344

Tablica 7. Komparativni rezultati za gripu tipa B.

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

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

	cobas® Influenza A/B & RSV (Roche)			
		+	-	Ukupno
VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit	+	22	4*	26
	-	3*	315	318
	Ukupno	25	319	344

Tablica 8. Komparativni rezultati za virus RSV.

Postotak podudarnosti pozitivnih rezultata je >84%, a postotak podudarnosti negativnih rezultata je >99 %.

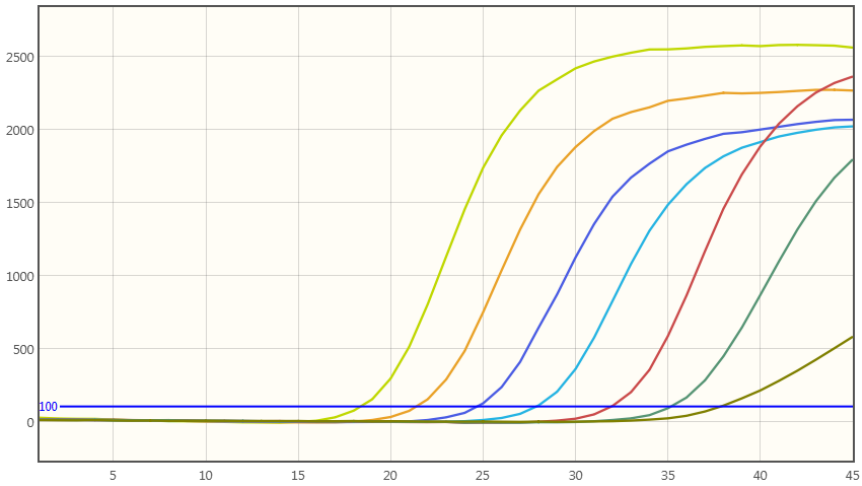
*Niska količina RNK predloška u ovom respiratornom uzorku je ispod granice detekcije za korištenu metodu.

Rezultati pokazuju visoku osjetljivost i specifičnost detekcije virusa gripe tipa A, gripe tipa B i/ili RSV pomoću kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit.

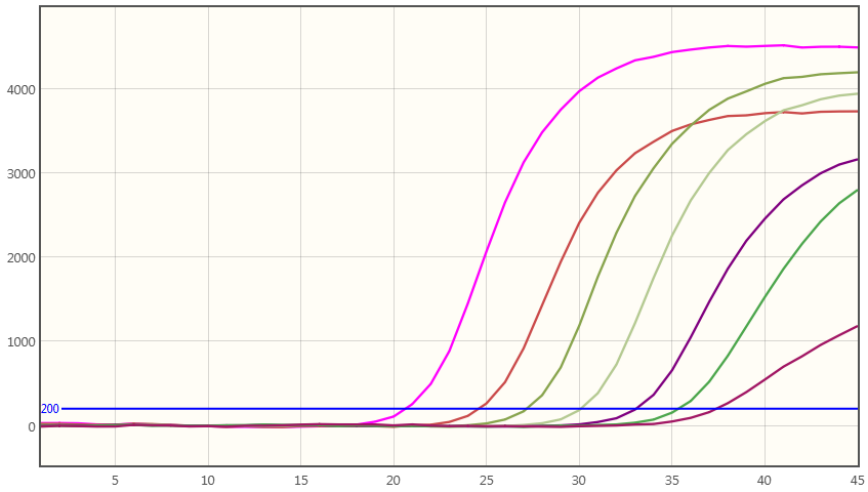
12.2. Analitička osjetljivost

Granica detekcije kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit iznosi ≥10 kopija RNK po reakciji za gripu tipa A, gripu tipa B i virus RSV s pozitivnom stopom od ≥95 % (Slika 2, 3 i 4).

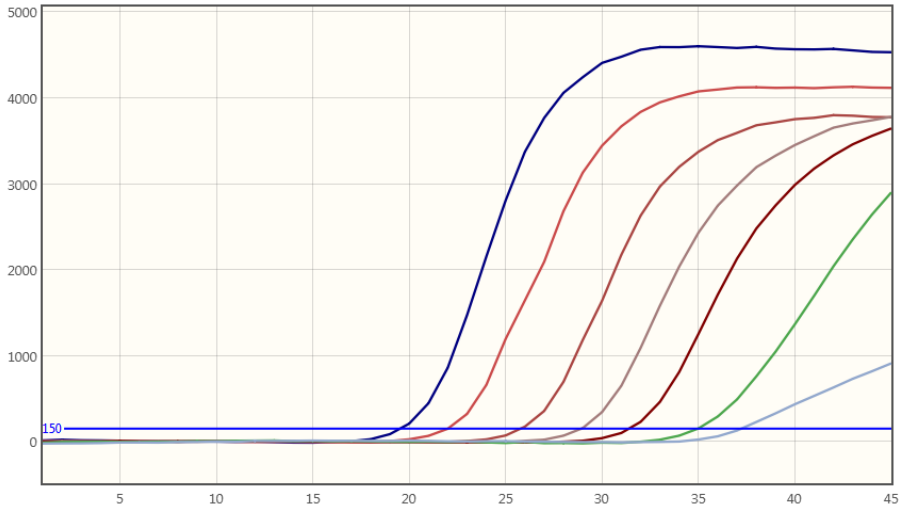
Slika 2. Serija razrjeđivanja predloška gripe tipa A ($2*10^6$-$2*10^1$ kopije/rxn) analizirana na BD MAX™ sustavu (475/520 (FAM) kanal).



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



Slika 4. Serija razrjeđivanja predloška virusa RSV ($2*10^6$-$2*10^0$ kopije/rxn) analizirana na BD MAX™ sustavu (630/665 (Cy5) kanal).



12.3. Analitička specifičnost

Specifičnost testa za gripu tipa A, gripu tipa B i virus RSV potvrđena je testiranjem panela koji se sastoji od različitih mikroorganizama koji predstavljaju najčešće respiratorne patogene. Nije zabilježena unakrsna reaktivnost između bilo kojih od sljedećih testiranih mikroorganizama, izuzev ciljanih patogena svakog testa:

Testiranje unakrsne reaktivnosti					
Bordetella pertussis	-	Ljudski rinovirus	-	A/Mallard/Netherlands/2/2009 (H7N7) virus	-/+
Bordetella parapertussis	-	Ljudski Adenovirus	-	Influenza A/Anhui/1/2013 (H7N9) virus	-/+
Legionella pneumophila	-	MERS Koronavirus	-	Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2)	-/+
Mycoplasma pneumoniae	-	Influenza A/New Caledonia/20/99(H1N1) virus	-/+	Influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2)	-/+
Streptococcus pneumoniae	-	Influenza A/California/7/2009(H1N1)pdm09-like virus	-/+	Influenza A/mallard/Netherlands/12/2000 (H7N7) - IBCDC-1	-/+
Staphylococcus aureus podsoj *aureus*	-	Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-/+	Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4	-/+
Staphylococcus aureus rezistentan na metilicin	-	Influenza A/Perth/16/2009(H3N2)-like virus	-/+	Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2)	-/+
Haemophilus influenzae MinnA	-	Influenza A/Thüringen/5/17 (H3N2) virus	-/+	Influenza A/South Australia/55/2014	-/+
Moraxella catarrhalis	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-/+	Influenza A/Uruguay/716/2007 (H3N2)(NYMC-175C)	-/+
Chlamydia psittaci genotip A i C	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-/+	Influenza B/Brisbane/60/2008-like virus	-/+
Chlamydophila pneumoniae	-	Influenza A/Turkey/Germany R2485+86/2014 (H5N8) virus	-/+	Influenza B/Florida/04/06 virus	-/+
Enterovirus 68 i 71	-	Influenza A/DE-SH/Reiherente/AR8444/ 2013 (H5N8) virus	-/+	Influenza B/Phuket/3073/2013 virus	-/+
Enterovirus Echovirus tipovi 11 i 30	-	A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1)	-/+	Influenza B/Colorado/6/2017	-/+
Virusi ljudske parainfluence tipa 1, 2, 3 i 4	-	A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a)	-/+	Influenza B/Maryland/15/2016	-/+
Ljudski metapneumovirus A i B	-	A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a)	-/+	Respiratorni sincicijski virus (RSV)	-/+
Ljudski koronavirus 229E	-	A/Hong Kong/213/2003 (H5N1) virus	-/+		

Tablica 9. Referentni patogeni mikroorganizmi korišteni u ovom ispitivanju.

12.4. Analitička reaktivnost

Reaktivnost kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit u realnom vremenu za gripu A procijenjena je u odnosu na sojeve: A/New Caledonia/20/99(H1N1) virus, A/California/7/2009(H1N1)pdm09-like virus, A/Michigan/45/2015 (H1N1)pdm09 virus, A/Perth/16/2009(H3N2)-like virus, A/Thüringen/5/17 (H3N2) virus, A/Switzerland/9715293/2013 (H3N2) virus, A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus, A/Turkey/Germany R2485+86/2014 (H5N8) virus, A/DE-SH/Reiherente/AR8444/ 2013 (H5N8) virus, A/Anhui/1/2013 (H7N9) virus, A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2), A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2), A/mallard/Netherlands/12/2000 (H7N7) - IBCDC-1, A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4, A/Singapore/INFIMH-16-0019/2016 (H3N2), A/South Australia/55/2014 and A/Uruguay/716/2007 (H3N2)(NYMC-175C), A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1) A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a), A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a), A/Hong Kong/213/2003 (H5N1) i A/Mallard/Netherlands/2/2009 (H7N7) virus, i pokazla je pozitivne rezultate.

Reaktivnost kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit u realnom vremenu za gripu B procijenjena je u odnosu na sojeve: B/Brisbane/60/2008-like virus (B/Victoria lineage), B/Florida/04/06 i B/Phuket/3073/2013 (B/Yamagata lineage), B/Colorado/6/2017, B/Maryland/15/2016, i pokazala je pozitivne rezultate.

Reaktivnost kompleta VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit u realnom vremenu za RSV procijenjena je u odnosu na humani respiratorni sincicijski virus (RSV A i B) i pokazala je pozitivne rezultate.

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14. Symbols for IVD components and reagents/ Símbolos para reakctivos i proizvodi i para diagnóstico *in vitro*.

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

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