VIASURE

Real Time PCR Detection Kits

by CerTest BIOTEC

Flu A, Flu B & RSV

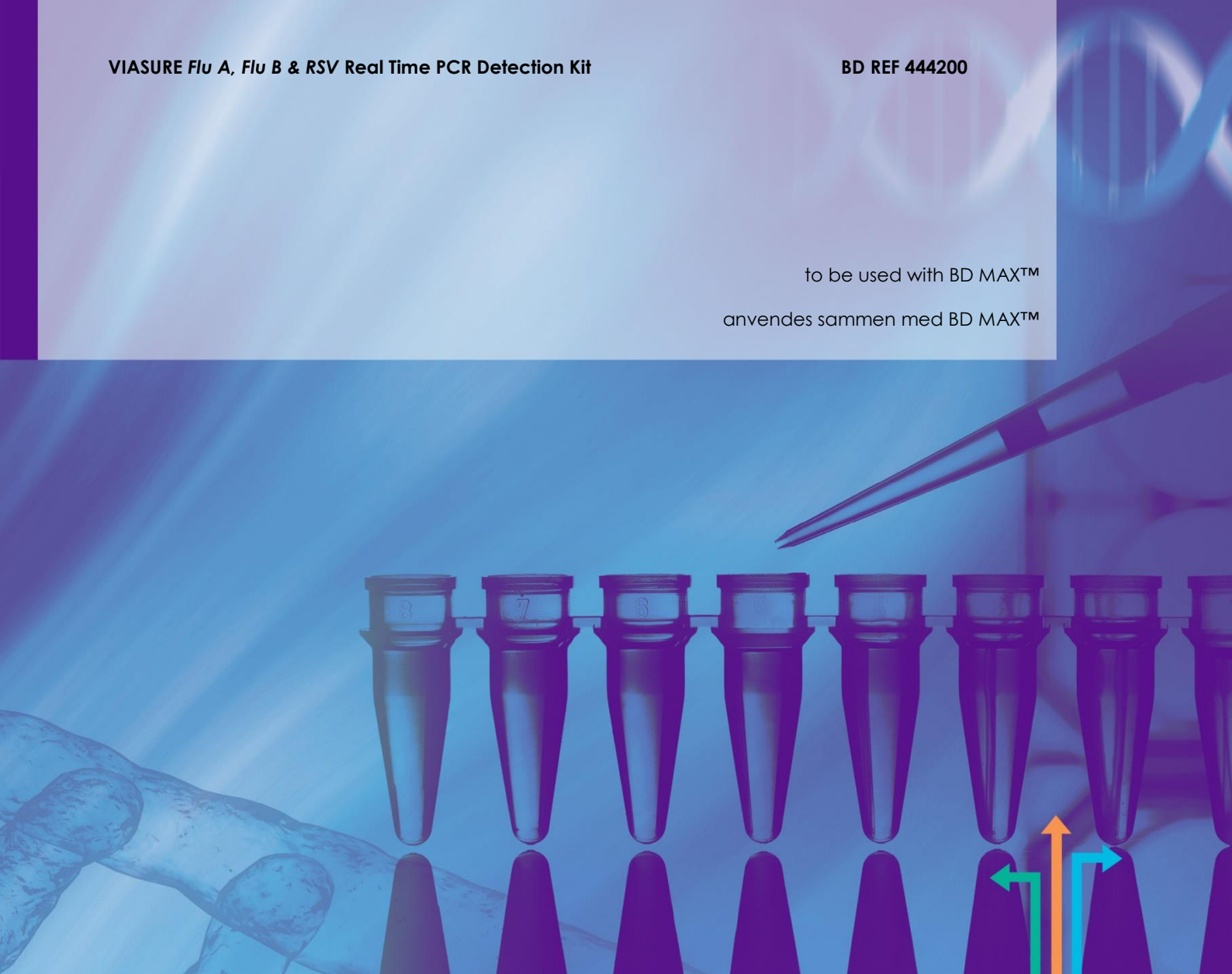
Handbook for the following references/

Håndbog for de følgende referencer

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

to be used with BD MAX™

anvendes sammen med 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 ≤-20ºC. The samples can be stored at 2 to 8ºC for up to 24 hours or frozen at -20ºC or -70ºC 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.

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

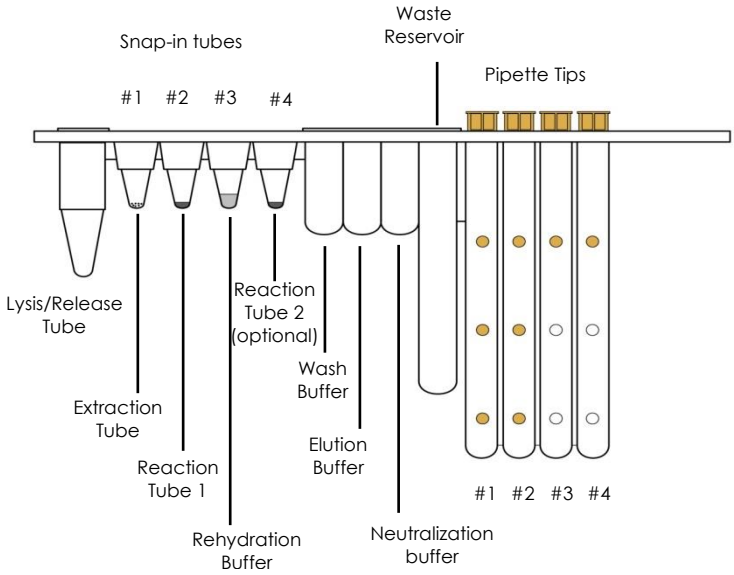


Figure 1. BD MAX™ TNA Reagent Strip (TNA) from the BD MAX™ ExK TNA-3 kit.

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 accured

-: No amplification accured

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.

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.*

		cobas® Influenza A/B & RSV (Roche)		
		+	-	Total
VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit	+	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.

12.1. 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 ($2*10^6$-$2*10^1$ copies/rxn) template run on the BD MAX™ System (475/520 (FAM) channel).

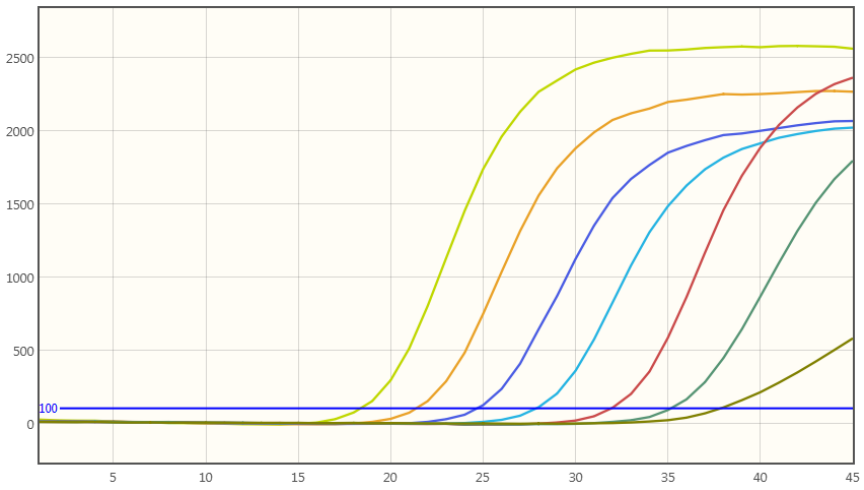


Figure 3. Dilution series of Flu B (2*10⁶-2*10¹ copies/rxn) template run on the BD MAX™ System (585/630 (ROX) channel).

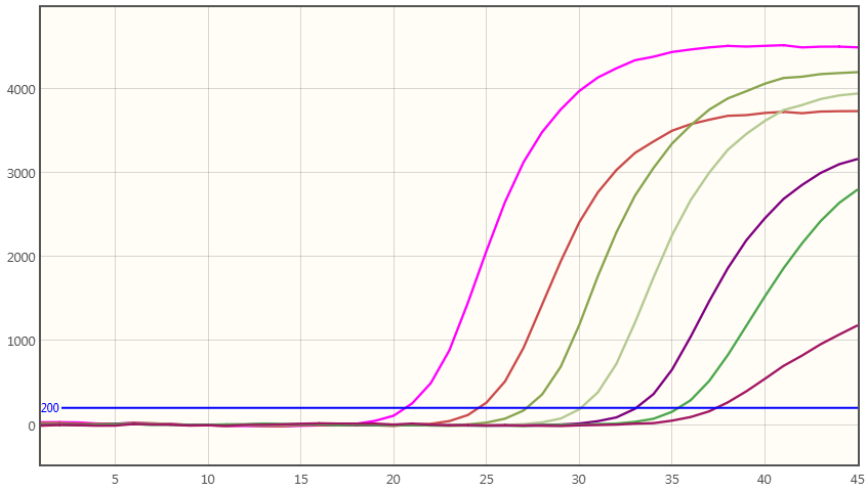
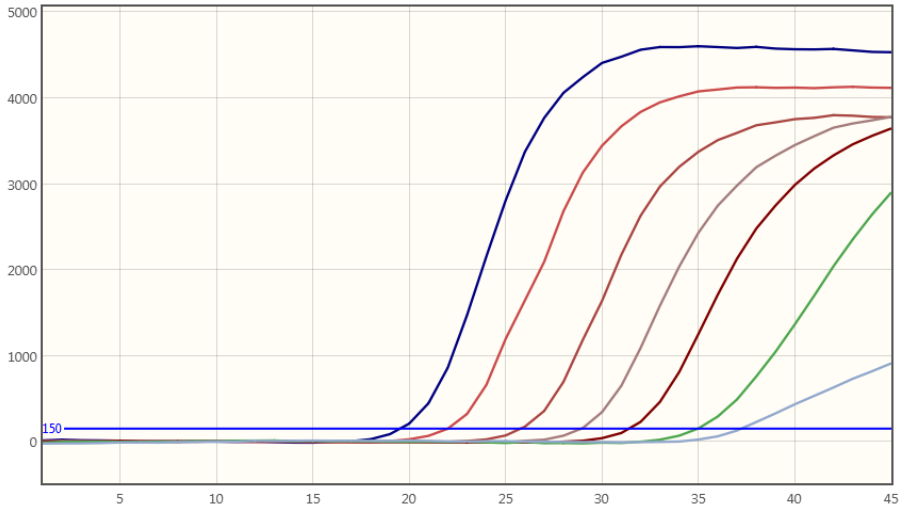


Figure 4. Dilution series of RSV (2*10⁶-2*10⁰ copies/rxn) template run on the BD MAX™ System (630/665 (Cy5) channel).



12.2. 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.

12.3. 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.

DANSK

1. Tilsigtet anvendelse

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit er beregnet til specifik identifikation og differentiering af influenza A, influenza B (Flu A og/eller B) og/eller human respiratorisk syncytialvirus (RSV) i luftvejsprøver fra patienter med tegn og symptomer på luftvejsinfektion. Denne test er beregnet som en hjælp til diagnosticering af influenza A, influenza B og/eller RSV kombineret med kliniske og epidemiologiske risikofaktorer. Analyserne bruger BD MAX™ systemet til ekstraktion af RNA og efterfølgende PCR i realtid, der anvender de medfølgende reagenser kombineret med universale reagenser og engangsartikler til BD MAX™ systemet. RNA fra kliniske præparater påvises ved hjælp af fluorescerende reporter-farveprober, der er specifikke for influenza A, influenza B og RSV.

2. Resumé og forklaring

Influenzavirusser hører til *Orthomyxoviridae*-familien og er årsag til størstedelen af de virusinfektioner, der forekommer i de nedre luftveje. Influenza A og B er en væsentlig årsag til sygelighed og dødelighed over hele verden, fordi ældre og svækkede personer har en særlig risiko for at udvikle svær sygdom og komplikationer såsom pneumoni. Patienter har nogle af disse symptomer eller alle: feber eller feberfornemmelse/kuldegysninger, hoste, ondt i halsen, tilstoppet næse og næseflåd, myalgi, hovedpine og nedsat appetit. Influenzavirusser kan spredes fra person til person på to forskellige måder: gennem luften (store dråber og aerosoler fra nys og hoste) og ved direkte eller indirekte kontakt.

Influenza A og B er kappeklædte, enkeltstrengede RNA-virusser, der indeholder otte segmenterede strenge af genom-RNA, som typisk koder for 11 eller 12 virusproteiner. Viruskappen, der stammer fra værtens plasmamembran, består af et dobbelt lipidlag, som indeholder transmembrane proteiner, såsom hæmagglutinin (HA) og neuraminidase (NA), og matrixproteinerne M1 og M2. Influenza A-virusser inddeles yderligere i undergrupper ud fra antigeniciteten i deres "HA-" og "NA-" molekyler, mens influenza B er inddelt i 2 antigent og genetisk adskilte slægter, Victoria og Yamagata.

Humane respiratoriske syncytialvirusser (RSV) hører til *Paramyxoviridae*-familien og er de vigtigste virusstoffer ved akutte luftvejsinfektioner. RSV er et kappeklædt, ikke-segmenteret, negativt, enkeltstrenget lineært RNA-genomvirus. Respiratorisk syncytialvirus er en almindelig medvirkende årsag til luftvejsinfektioner som bronchitis, pneumoni og kronisk obstruktiv lungesygdom hos mennesker i alle aldre. Patienter har ofte nogle af disse symptomer eller alle: næseflåd, lav feber, hoste, ondt i halsen, hovedpine og hvæsende vejrtrækning. RSV overføres via store dråber nasopharyngealt sekret fra inficerede personer, tæt kontakt eller selvinokulation efter berøring af kontaminerede overflader.

Det kan være vanskeligt at diagnosticere, da en lang række patogener kan forårsage akutte luftvejsinfektioner, som giver de samme kliniske syndromer. Det er påvist, at PCR-analyse i realtid er et følsomt og specifikt diagnostisk værktøj til påvisning af influenza A-, influenza B- og RSV-virusser.

3. Procedureprincip

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit er beregnet til diagnosticering af influenza A, influenza B og/eller RSV i luftvejsprøver. Påvisningen foretages i et ettrins-, realtids- RT-format, hvor revers transkription og den efterfølgende amplifikation af den specifikke målsekvens sker i det samme reaktionsrør. Det isolerede RNA-mål transkriberes ved at generere komplementært DNA via revers transkriptase, som følges af amplifikation af et konserveret område af *M1*-genet for influenza A og influenza B og et konserveret område af *N*-genet for RSV ved hjælp af specifikke primere og fluorescens-mærkede prober.

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit er baseret på DNA-polymerases 5´ exonuclease-aktivitet. Ved DNA-amplifikation kløver dette enzym den probe, der er bundet til den komplementære DNA-sekvens, og adskiller quencher-farven fra reporter-farven. Denne reaktion genererer en forøgelse af fluorescenssignalet, som er proportional med kvantiteten af målskabelonen. Denne fluorescens kan måles på BD MAX™ systemet.

Hvert rør i VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit indeholder alle de komponenter, der er nødvendige til realtids-PCR-analyse (specifikke primere/prober, dNTP'er, buffer, polymerase, revers transkriptase), i et stabiliseret format, samt intern kontrol til monitorering af PCR-hæmning. Influenza A RNA-mål forstærkes og påvises i kanal 475/520, influenza B RNA i kanal 585/630, RSV RNA i kanal 630/665 og den interne kontrol (IC) i kanal 530/565.

4. Medfølgende reagenser

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit indeholder de følgende materialer og reagenser, som beskrevet i tabel 1:

Reference	Reagens/materiale	Beskrivelse	Farve	Antal
VS-ABR212R	*Flu A, Flu B & RSV* reaktionsrør	En blanding af enzymer, primere, prober, buffer, dNTP'er, stabilisatorer og intern kontrol i stabiliseret format	Transparent rød folie	2 poser a 12 rør
VS-RB05	Rør med rehydreringsbuffer	Opløsning til rekonstitution af det stabiliserede produkt	Transparent lilla folie	1 pose a 24 rør

Tabel 1. Reagenser og materialer, der medfølger i VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit med ref. VS-ABR124.

5. Reagenser og udstyr, som skal fremskaffes af brugeren

Den følgende liste indeholder de materialer og det udstyr, der kræves til brugen, men som ikke medfølger i VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit.

- PCR-instrument i realtid: BD MAX™ System.
- BD MAX™ ExK™ TNA-3 (ref: 442828)
- BD MAX™ PCR Cartridges (ref: 437519)
- Vortex.
- Mikropipetter (nøjagtighed på 2-1000 µL).

- Filterspidser.
- Pudderfrie engangshandsker.

6. Transport og opbevaringsforhold

- Kittene kan sendes og opbevares ved 2-40 °C indtil den udløbsdato, der er angivet på etiketten.
- Komponenterne må ikke udsættes for sollys.

7. Forholdsregler for brugere

- Til professionel in vitro-diagnostisk brug.
- Anvend ikke reagenser og/eller materialer, der er for gamle.
- Analysekittet må ikke bruges, hvis etiketten, som forsegler den ydre emballage, er brudt.
- Reagenserne må ikke bruges, hvis beskyttelsesæsken har været åbnet eller er beskadiget ved modtagelsen.
- Reagenser må ikke bruges, hvis de beskyttende poser er åbne eller defekte ved modtagelse.
- Reagenserne må ikke anvendes, hvis tørremidlet ikke er i poserne eller er ødelagt.
- Fjern ikke tørremidlet fra reagensposerne.
- De beskyttende poser med reagenser skal straks lukkes til med lynlåsen efter hver brug. Fjern eventuel overskydende luft i poserne, inden de lukkes.
- Anvend ikke reagenser, hvis folien er brudt eller beskadiget.
- Bland ikke reagenser fra forskellige poser og/eller kit og/eller lotnumre.
- Beskyt reagenser mod fugt. Længerevarende udsættelse for fugt kan have en negativ indflydelse på produktets ydelse.
- Hvis der udføres PCR-test i det samme laboratorieområde, skal der udvises forsigtighed for at sikre, at VIASURE Flu A, Flu B & RSV Real Time PCR Detection kit, BD MAX™ ExK™ TNA-3 ekstraktionskit, de nødvendige reagenser til testning og BD MAX™ systemet ikke kontamineres. Der skal skiftes handsker inden håndtering af reagenser og kassetter.
- Konstruer en ensrettet arbejdsgang. Den bør begynde i ekstraktionsområdet og derefter fortsætte til amplifikations- og påvisningsområdet. Lad ikke prøver, udstyr og reagenser komme tilbage til det område, hvor det forrige trin blev udført.
- Følg god laboratoriepraksis. Benyt beskyttelsestøj, engangshandsker, beskyttelsesbriller og maske. Der må ikke spises, drikkes eller ryges i arbejdsområdet. Vask hænder, når testen er færdig.
- Præparater skal behandles som potentielt smittefarlige, og det samme gælder alle reagenser og materialer, der har være eksponeret for prøverne. Desuden skal de håndteres i henhold til nationale sikkerhedsregler. Træf de nødvendige forholdsregler under indsamling, opbevaring, behandling og bortskaffelse af prøver.
- Regelmæssig dekontaminering af almindeligt anvendt udstyr anbefales, især mikropipetter og arbejdsflader.
- Se brugervejledningen til BD MAX™ systemet for yderligere advarsler, forholdsregler og procedurer.

8. Testprocedure

8.1. PRØVETAGNING, -OPBEVARING OG -TRANSPORT

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit er blevet valideret på podninger, som blev indsamlet med fleksible nasopharyngeale podepinde med nylonflock og straks anbragt i virustransportmedium (Vircell, Spanien). Yderligere luftvejspræparater fra symptomatiske patienter kan testes ifølge litteraturen (dvs. nasale/dybnasale/ nasopharyngeale podninger, kombinerede næse- og halspodninger, nasopharyngale/nasale/trakeale aspirater, nasopharyngale/nasale/hals-skylninger, bronchoalveolær lavage, sputum), men skal valideres af brugeren.

Indsamling, opbevaring og transport af præparater skal ske under de forhold, der er valideret af brugeren. Generelt skal luftvejsprøver indsamles og mærkes korrekt i rene beholdere med eller uden transportmedium (afhængigt af prøvetypen) og behandles hurtigst muligt for at garantere testens kvalitet. Præparaterne skal transporteres i henhold til lokale og nationale regler for transport af patogent materiale. Ved langvarig transport (over 24 timer) anbefaler vi forsendelse ved ≤-20 °C. Prøverne kan opbevares ved 2-8 °C i op til 24 timer eller frosne ved -20 °C eller -70 °C ved langtidsopbevaring. Gentagne frysnings-optøningscyklusser bør undgås for at hindre forringelse af prøven og nukleinsyrerne.

8.2. KLARGØRING AF PRØVER OG RNA-EKSTRAKTION

Klargør prøven ifølge anbefalingerne i brugsanvisningen til det anvendte ekstraktionskit, BD MAX™ ExK™ TNA-3. Bemærk, at andre præparater kan kræve forbehandling. Procedurer for klargøring til ekstraktion for den specifikke anvendelse skal udarbejdes og valideres af brugeren.

1. Pipettér 200-400 µL af det kliniske luftvejspræparat ned i et BD MAX™ TNA-3-prøvebufferrør, og luk røret med en membranhætte. Sørg for at blande præparatet helt ved hjælp af vortex ved høj hastighed i ét (1) minut.

2. Fortsæt til Betjening af BD MAX™ systemet.

8.3. TESTPROTOKOL

Bemærk: Se brugermanualen til BD MAX™ systemet for at få detaljerede instruktioner.

8.3.1. Oprettelse af PCR-testprogram for VIASURE Flu A, Flu B & RSV Real Time PCR Detection kit

Bemærk: Hvis du allerede har oprettet testen for VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit, kan du springe trin 8.3.1 over og gå direkte til 8.3.2.

1) Vælg fanen "Test Editor" (Testeditor) på skærmbilledet "Run" (Kørsel) i BD MAX™ systemet.
2) Klik på knappen "Create" (Opret).
3) Navngiv testen i vinduet "Test Name" (Testnavn). dvs. VIASURE *Flu A, Flu B & RSV*.
4) Vælg "ExK TNA-3" i rullemenuen "Extraction Type" (Ekstraktionstype).
5) Vælg "Type 5" i rullemenuen "Master Mix Format" (Mastermix format).

a. Bemærk: Produktet kan anvendes i kombination med endnu en Viasure for BD MAX test. I så fald skal du vælge "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Dobbelt mastermix koncentreret frysetørret MM med rehydreringsbuffer (type 5)).

6) Vælg "User defined" (Brugerdefineret) i "Sample extraction parameters" (Prøveekstraktionsparametre), og juster prøvevolumen til det anvendte kliniske præparats volumen plus 550 µL.

a. Eksempel: Hvis der pipetteres 200 µL af det kliniske luftvejspræparat ned i et BD MAX TNA-3-prøvebufferrør, skal parameteren sættes til 750 µL.

b. Bemærk: Maksimumindstillingen er 950 µL.

7) Vælg "Call Ct at Threshold Crossing" (Fastlæg Ct ved tærskelskæring) i "Ct Calculation" (Ct-beregning).

8) På fanen "PCR settings" (PCR-indstillinger) skal du indtaste følgende parametre: "Channel Settings" (Kanalindstillinger), "Gains" (Forstærkning) og "Threshold" (Tærskel) (tabel 2).

a. Bemærk: Produktet kan anvendes i kombination med endnu en Viasure for BD MAX test. I så fald skal PCR-indstillinger og testtrin udføres for positionerne 2 (grøn) og 4 (blå).

Channel (Kanal)	Alias	Gain (Forstærkning)	Threshold (Tærskel)	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

Tabel 2. PCR-indstillinger.

9) På fanen "PCR Settings" (PCR-indstillinger) skal du indtaste følgende parametre "Spectral Cross Talk" (Spektral krydstale) (tabel 3) samt

		False Receiving Channel (Kanal, der modtager falsk input)				
	Channel (Kanal)	475/520	530/565	585/630	630/665	680/715
Excitation Channel (Excitationskanal)	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	-

Tabel 3. Spectral cross-talk parameters (Spektrale krydstaleparametre).

10) På fanen "Test Steps" (Testtrin) skal du indtaste PCR-protokollen (tabel 4).

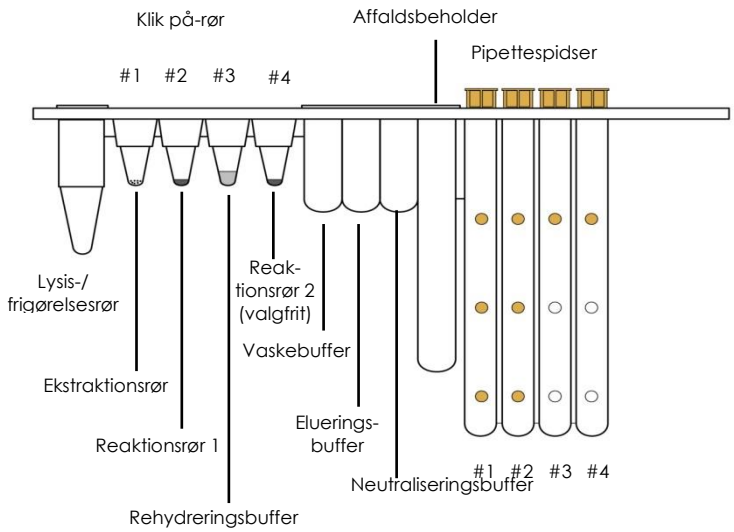
Navn på trin	Profile Type (Profiltype)	Cycles (Cyklusser)	Time (s) (Tid (s))	Temperatur	Detect (Påvis)
Revers transkription	Hold	1	900	45 °C	-
Indledende denaturering	Hold	1	120	98 °C	-
Denaturering og hærdning/forlængelse (dataindsamling)	2-temperatur	45	10	95 °C	-
			61.1	63 °C	✓

Tabel 4. PCR-protokol.

11) Klik på knappen "Save Test" (Gem test)

8.3.2. BD MAX™ stativopsætning

1) For hvert præparat, der skal testes, fjernes der en separat reagensstrimmel (BD MAX™ TNA Reagent Strip (TNA)) fra BD MAX™ ExK TNA-3 kittet. Bank forsigtigt hver strimmel mod en hård overflade for at sikre, at væskerne befinder sig i bunden af rørene, og sæt den i BD MAX™ systemets prøvestativ.
2) Fjern det nødvendige antal BD MAX™ ExK™ TNA-ekstraktionsrør (B4) (hvid folie) fra beskyttelsesposen. Klik ekstraktionsrøret/-rørene (hvidt folie) på plads i de tilsvarende positioner i TNA (position 1, hvid farvekode på stativet. Se figur 1). Fjern overskydende luft, og luk posen vha. lynlåsen.
3) Fastsæt og adskil det relevante antal VIASURE *Flu A, Flu B & RSV*-reaktionsrør (rød folie), og klik dem på plads i de tilsvarende positioner i strimlen (position 2, grøn farvekode på stativet. Se figur
    a. Fjern overskydende luft, og luk aluminiumsposerne med lynlåsen.
    b. Rehydreringen udføres korrekt ved at sørge for, at det frysetørrede produkt ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen.
        i. Bemærk: Hvis du vælger formatet "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Dobbelt mastermix koncentreret frysetørret MM med rehydreringsbuffer (type 5)) (afsnit 8.3.1), skal du fastsætte og adskille det relevante antal VIASURE-reaktionsrør (forskellig folie) og klikke dem på plads i de tilsvarende positioner i strimlen (position 4, blå farvekode på stativet. Se figur 1). Fjern overskydende luft, og luk aluminiumsposerne med lynlåsen.
4) Fjern det nødvendige antal rehydreringsbufferrør (lilla folie), og klik dem på plads i de tilsvarende positioner i strimlen (position 3, ingen farvekode på stativet. Se figur 1). Fjern overskydende luft, og luk posen med lynlåsen.
    a. Overførslen udføres korrekt ved at sørge for, at væsken ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen.



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

8.3.3. Opsætning af BD MAX™ instrumentet

1) Vælg fanen "Work List" (Arbejdsliste) på skærmbilledet "Run" (Kørsel) i BD MAX™ systemets software v4.50A eller nyere.
2) Vælg VIASURE *Flu A, Flu B & RSV* i rullemenuen "Test" (hvis den ikke allerede er oprettet, se afsnit 8.3.1).

3) Vælg det relevante kitlotnummer (findes på den ydre emballage til det ekstraktionskit, der anvendes) i rullemenuen (valgfrit).
4) Angiv identifikationsnummeret for prøvebufferrøret i vinduet Sample tube (Prøverør) i arbejdslisten ved enten at scanne stregkoden med scanneren eller ved at indtaste oplysningerne manuelt.
5) Indtast præparat-id og patient-id i vinduet "Accession" (Adgang) i "Worklist" (Arbejdsliste) (hvis det er relevant), og klik på knappen "Save" (Gem). Fortsæt, indtil alle prøvebufferrør er indtastet. Kontrollér, at præparat-id/patient-id og prøvebufferrørene er korrekt kombineret.
6) Placér det klargjorte prøvebufferrør i BD MAX™ stativet.
7) Sæt stativet/stativerne i BD MAX™ systemet (stativ A placeres i venstre side af BD MAX™ systemet og stativ B i højre side).
8) Anbring det påkrævede antal BD MAX™ PCR-kassetter i BD MAX™ systemet.
9) Luk BD MAX™ systemets låge.
10) Klik på "Start Run" (Start kørsel) for at begynde proceduren.

8.3.4. BD MAX™ rapport

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

9. Tolkning af resultater

Se brugervejledningen til BD MAX™ systemet for at få en detaljeret beskrivelse af dataanalysen.

Dataanalysen udføres af BD MAX™ softwaren i overensstemmelse med fabrikantens anvisninger. BD MAX™ softwaren rapporterer Ct-værdier og amplifikationskurver for hver detektorkanal for hver prøve, der blev testet, på følgende måde:

- En Ct-værdi på 0 angiver, at der ikke blev beregnet nogen Ct-værdi af softwaren med den specifikke tærskel (se tabel 4). En amplifikationskurve for prøven, som viser Ct-værdien "0", skal kontrolleres manuelt.

- En Ct-værdi på -1 angiver, at der ikke har været nogen amplikationsproces.

- Enhver anden Ct-værdi skal tolkes i korrelation med amplifikationskurven og i henhold til retningslinjerne for tolkning af prøver som beskrevet i tabel 5.

Tjek det interne kontrolsignal for at kontrollere, at amplifikationsblandingen fungerer korrekt. Tjek desuden, at der ikke er nogen rapport om BD MAX™ systemfejl.

Brug nedenstående tabel til at læse og analysere resultaterne:

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	**Resultatet Unresolved (uløst) (UNR), som opnås under tilstedeværelse af inhibitorer i PCR-reaktionen eller når der opstår et overordnet problem (der ikke rapporteres med en fejlkode) under prøvebehandlingen og/eller amplifikationstrinnene.**
IND	IND	IND	IND	**Analyseresultatet er Indeterminate (ubestemmeligt) (IND). Skyldes en fejl i BD MAXTM-systemet. Analyseresultat, der vises i tilfælde af en instrumentfejl, der knyttet til en fejlkode.**
INC	INC	INC	INC	**Analyseresultatet er Incomplete (ufuldstændigt) (INC). Skyldes fejl i BD MAXTM-systemet. Analyseresultatet vises, hvor en fuldstændig kørsel ikke kunne gennemføres.**

Tabel 5. Tolkning af prøver

+: Amplifikationskurve

-: Ingen amplifikationskurve

En prøve anses for positiv, hvis den opnåede Ct-værdi er under 40. Den interne kontrol kan vise et amplifikationssignal, men gør det ikke nødvendigvis, da et højt kopiantal for målet kan forårsage amplifikation fortrinsvis af målspecifikke nukleinsyrer i stedet for den interne kontrol. I sådanne tilfælde er påvisning af den interne kontrol ikke nødvendig.

En prøve anses for negativ, hvis den ikke viser noget amplifikationssignal i påvisningssystemet, og den interne kontrol er positiv. Hæmning af PCR-reaktionen kan udelukkes via amplifikationen af den interne kontrol.

I tilfælde af uafklarede resultater, fravær af signal fra intern kontrol i en negativ prøve, anbefaler vi at gentage analysen med en fortyndet prøve 1:10 for at tjekke for mulige hæmningsproblemer.

10. Testens begrænsninger

- Testresultaterne bør vurderes af sundhedspersonalet på baggrund af anamnese, kliniske symptomer og andre diagnostiske test.
- Selvom denne analyse kan foretages med andre typer prøver, er den blevet valideret med halspodninger.
- Testens kvalitet afhænger af kvaliteten af prøven. Der skal ekstraheres passende RNA fra kliniske prøver. Forkert indsamling, opbevaring og/eller transport af præparater kan give falsk negative resultater.

- Der kan blive påvist ekstremt lave målniveauer under påvisningsgrænsen, men resultaterne er muligvis ikke reproducerbare.
- Der er mulighed for falsk positive resultater, som skyldes krydskontaminering med influenza A, influenza B og/eller RSV, enten ved prøver, der indeholder høje koncentrationer af mål-RNA, eller ved kontaminering, som skyldes PCR-produkter fra tidligere reaktioner.
- Resultaterne, der indhentes med VIASURE Flu A, Flu B & RSV Real Time PCR Detection kit kan være Unresolved (uløste), hvis prøven indeholder inhibitorer eller røret med frysetørret reaktionsblanding ikke blev korrekt rehydreret, eller de kan være Indeterminate (ubestemmelige) eller Incomplete (ufuldstændige) som følge af instrumentfejl, og således kræve, at testen gentages.

11. Kvalitetskontrol

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection Kit indeholder en intern kontrol i hvert reaktionsrør, der bekræfter, at teknikken fungerer korrekt.

12. Funktionsdata

12.1. Klinisk sensitivitet og specificitet

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kittets kliniske funktion blev testet med 344 luftvejspræparater (halspodninger) fra symptomatiske patienter. Disse resultater blev sammenlignet med resultater opnået med en molekylær påvisningsmetode (cobas® Influenza A/B & RSV (Roche)).

Resultaterne var følgende:

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

Tabel 6. Sammenligningsresultater for influenza A. Positiv procentvis overensstemmelse er >98 % og negativ procentvis overensstemmelse er >96 %.

**Den lave mængde skabelon-RNA i denne luftvejsprøve ligger under påvisningsgrænsen for den anvendte metode.*

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

Tabel 7. Sammenligningsresultater for influenza B. Positiv procentvis overensstemmelse er >96 % og negativ procentvis overensstemmelse er >99 %.

**Den lave mængde skabelon-RNA i denne luftvejsprøve ligger under påvisningsgrænsen for den anvendte metode.*

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

Tabel 8. Sammenligningsresultater for RSV. Positiv procentvis overensstemmelse er >84 % og negativ procentvis overensstemmelse er >99 %.

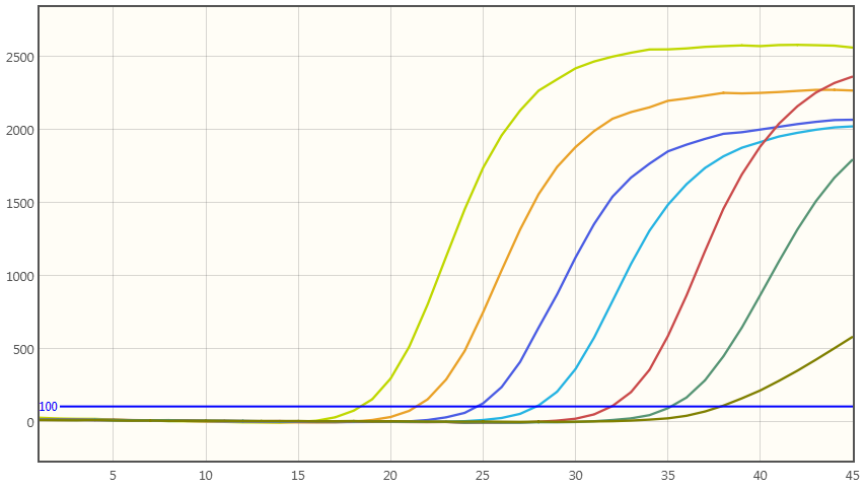
**Den lave mængde skabelon-RNA i denne luftvejsprøve ligger under påvisningsgrænsen for den anvendte metode.*

Resultaterne viser høj sensitivitet og specificitet til påvisning af influenza A-, influenza B- og/eller RSV-virusser med VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit.

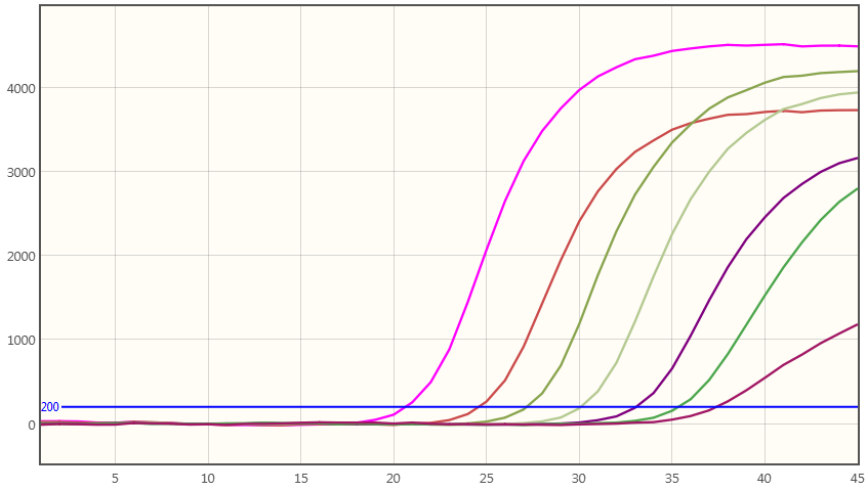
12.2. Analytisk sensitivitet

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kit har en påvisningsgrænse på ≥10 RNA-kopier pr. reaktion for influenza A, influenza B og RSV med en positiv forekomst på ≥95 % (figur 2, 3 og 4).

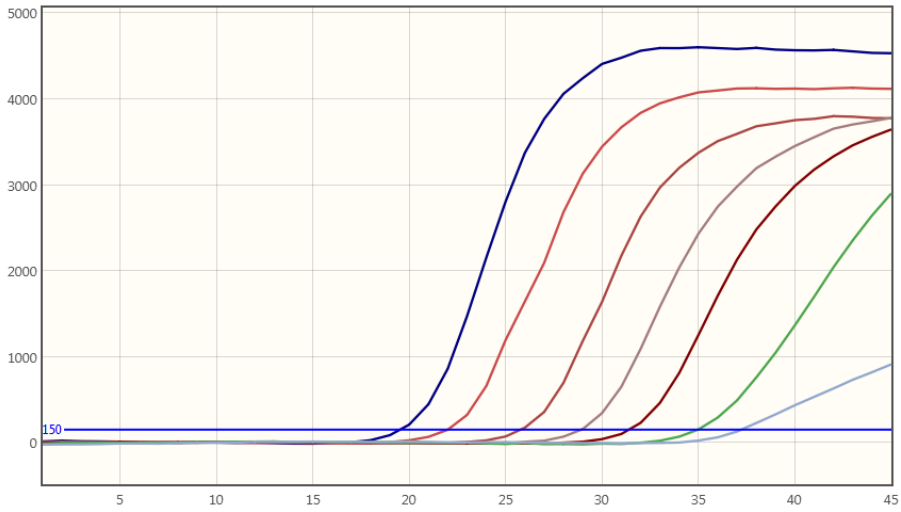
Figur 2. Fortyndingsrække for influenza A ($2*10^6$-$2*10^1$ kopier/reaktion). Skabelon kørt på BD MAX™ systemet (475/520 (FAM) kanal).



Figur 3. Fortyndingsrække for influenza B ($2*10^6$-$2*10^1$ kopier/reaktion). Skabelon kørt på BD MAX™ systemet (585/630 (ROX) kanal).



Figur 4. Fortyndingsrække for RSV ($2*10^6$-$2*10^0$ kopier/reaktion). Skabelon kørt på BD MAX™ systemet (630/665 (Cy5) kanal).



12.3. Analytisk specificitet

Specificiteten for analysen af influenza A, influenza B og RSV blev bekræftet ved test af et panel bestående af forskellige mikroorganismer, som repræsenterede de mest almindelige luftvejspatogener.

Der blev ikke påvist nogen krydsreaktivitet mellem nogen af følgende testede mikroorganismer bortset fra de målrettede patogener fra hvert assay:

Krydsreaktivitet Test					
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	-/+		

Tabel 9. Patogene referencemikroorganismer anvendt i denne undersøgelse.

12.4. Analytisk reaktivitet

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kittets reaktivitet for influenza A blev vurderet i forhold til disse stammer: 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) og A/Mallard/Netherlands/2/2009 (H7N7) virus, med positive resultater.

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kittets reaktivitet for influenza B blev vurderet i forhold til disse stammer: B/Brisbane/60/2008-like virus (B/Victoria lineage), B/Florida/04/06 og B/Phuket/3073/2013 (B/Yamagata lineage), B/Colorado/6/2017, B/Maryland/15/2016 med positive resultater.

VIASURE *Flu A, Flu B & RSV* Real Time PCR Detection kittets reaktivitet for RSV blev vurderet i forhold til human respiratorisk syncytialvirus (RSV A og B) med positive resultater.

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14. Symbols for IVD components and reagents/Symboler for IVD-komponenter og reagenser

IVD	*In vitro* diagnostic device In vitro diagnostisk anordning		Keep dry Opbevares tørt		Use by Anvendes inden		Manufacturer Fabrikant
LOT	Batch code Batchkode		Consult instructions for use Se brugsan-visningen		Temperature limitation Tempera-turgrænse	Σ	Contains sufficient for <n> test Indeholder tilstrækkeligt til <n> prøver
DIL	Sample diluent Prøvediluent	REF	Catalogue number Katalognummer				

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F-362 rev01

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Real Time PCR Detection Kits

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