VIASURE

Real Time PCR Detection Kit

Pneumocystis jirovecii

for BD MAX™ System

CE IVD

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

PRODUCT / PRODUKT	REFERENCE / REFERENCE
VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System	444207 / VS-JIR124

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

Content

Indholdsfortegnelse

ENGLISH

1. Intended use

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System is an automated real-time PCR test designed for the qualitative detection of *Pneumocystis jirovecii* DNA in respiratory samples (bronchoalveolar lavage) from patients suspected of respiratory infection by their healthcare professional (HCP). This test is intended to be used as an aid in the identification of *Pneumocystis jirovecii* in combination with patient's clinical signs and symptoms and epidemiological risk factors. The assay uses the BD MAX™ System for automated extraction of DNA and subsequent real-time PCR, employing the reagents provided combined with universal reagents and disposables for the BD MAX™ System. DNA from respiratory samples is detected using fluorescent reporter dye probes specific for *Pneumocystis jirovecii*.

2. Summary and Explanation

Pneumocystis jirovecii pneumonia (PCP) is an acute and life-threatening lung disease caused by the fungus *Pneumocystis jirovecii*. PCP is an important disease of immunocompromised humans, particularly patients with HIV, but also patients with an immune system that is severely suppressed for other reasons. In humans with a normal immune system, it is an extremely common silent infection. In developing regions of the world, the prevalence of PCP was once thought to be much lower, but studies have shown that the lower reported incidence is likely a failure to accurately diagnose.

The symptoms of PCP are nonspecific, in patients with HIV tends to present much later, often after several weeks of symptoms, compared with PCP associated with other immunocompromising conditions. Symptoms of PCP include the following: progressive exertional dyspnea, fever, non-productive cough, chest discomfort, weight loss, chills and hemoptysis (rare).

PCP is difficult to diagnose as a result of the associated nonspecific signs and symptoms. Because *P. jirovecii* cannot be propagated in culture, microscopic visualization of cysts or trophic forms in pulmonary specimens with cytochemical or immunofluorescent staining with monoclonal antibodies and/or DNA amplification are the standard procedures to detect this microorganism.

3. Principle of the procedure

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System is designed for the qualitative detection of DNA from *Pneumocystis jirovecii* in respiratory samples. After DNA isolation, the identification of *Pneumocystis jirovecii* is performed by the amplification of a conserved region of the large-subunit (mt LSU) rRNA gene using specific primers and a fluorescent-labelled probe.

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System is based on 5´ exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent

signal which is proportional to the quantity of the target template. This fluorescence is measured on the BD MAX™ System.

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System contains in each tube all the components necessary for real-time PCR assay (specific primers/probes, dNTPs, buffer, polymerase) in a stabilized format, as well as an internal control to monitor the extraction process and/or inhibition of the polymerase activity.

Target	Channel	Gene
Pneumocystis jirovecii	475/520	Large-subunit (mt LSU) rRNA gene
Internal control (IC)	530/565	-

Table 1. Target, channel and genes.

4. Reagents provided

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System includes the following materials and reagents detailed in Table 2:

Reagent/Material	Description	Color/ Barcode	Amount
Pneumocystis jirovecii reaction tube	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and internal control in stabilized format	Green or 1D foil	2 pouches of 12 transparent tubes
Rehydration Buffer tube	Solution to reconstitute the stabilized product	11 foil	1 pouch of 24 transparent tubes

Table 2. Reagents and materials provided in VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System with Cat. N°. VS-JIR124 (444207).

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 *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System.

- Real-time PCR instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442828 or 442827).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).
- N-Acetyl-L-cysteine (recommended N-Acetyl-L-cysteine Ref. A7250, Merck KGaA).
- Nuclease-free water.
- Filter tips.
- Powder-free disposable gloves.

6. Transport and storage conditions

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

7. Precautions for users

- The product is intended for use by professional users only, such as laboratory or health care professionals and technicians, trained in molecular biological techniques.
- For *in vitro* diagnostic use.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective box is open or broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Protect reagents from humidity. Prolonged exposure to humidity may affect product performance.
- Keep components away from light.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, any additional reagents required for testing and the BD MAX™ System are not contaminated. Always avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges (BD MAX™ PCR Cartridge).
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridge are designed to prevent contamination.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles and mask. Do not eat, drink, smoke or apply cosmetic products in the working area. Wash your hands after finishing the test.
- Samples must be treated as potentially infectious and/or biohazardous, as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national safety regulations. Take necessary precautions during the collection, transport, storage, handling, and disposal of samples.
- Samples and reagents must be handled in a biological safety cabinet. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Dispose of waste in compliance with local and state regulations.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- In accordance with Regulation (EC) No 1907/2006 (REACH), VIASURE Real Time PCR Detection Kits do not require Material Safety Data Sheets on account of their classification as non-hazardous to health and the environment, because they do not contain substances and/or mixtures which meet the hazard classification

criteria available in Regulation (EC) No 1272/2008 (CLP), or which are in concentrations higher than the value established in the mentioned regulation for their declaration.

- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Test procedure

8.1. Sample collection, storage and transport

The VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System has been tested on bronchoalveolar lavages (BALs). Other types of samples must be validated by the user.

Collection, storage and transport of specimens should be maintained per the conditions validated by the user. Overall, respiratory samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type) and processed as soon as possible to guarantee the quality of the test. The specimens should be transported at 4°C for up to 7 days, following the local and national regulations for the transport of pathogen material. For long term transport (more than 7 days), we recommend shipping at ≤-20°C or lower. It is recommended to use fresh specimens for the test. The samples can be stored at 4°C for up to 7 days or frozen at -20°C or ideally at -80°C for conservation. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

The respiratory samples must be collected, transported and stored according to appropriate laboratory guidelines. For details, refer to the CDC guideline (Specimen collection guidelines. Website https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf) and the IDSA guideline (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94).

If sputum samples are used, they can be tested according to recommendations cited below.

8.2. Sample preparation and DNA extraction

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

1. Pipette 200 µL of BAL into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

2. For sputum samples, add acetylcysteine (recommended N-Acetyl-L-cysteine Ref. A7250, Merck KGaA) to the sample at a 1:1 ratio (i.e. 250 µl of sputum and 250 µl of acetylcysteine 100 mg/ml), mix by vortexing and heat 95°C for 10 minutes. Pipette 200 µl of the pretreated sputum into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

8.3. PCR protocol

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

8.3.1. Creating PCR test program for VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System

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

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

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	*P. jirovecii*	50	200	0	33*
530/565 (HEX)	IC	80	200	0	35
585/630 (ROX)	-	0	0	0	0
630/665 (Cy5)	-	0	0	0	0
680/715 (Cy5.5)	-	0	0	0	0

Table 3. PCR settings.

*** The use of a clinical threshold of Ct 33 in this test system (equalling 3000 copies/ml) allows to distinguish between high and low fungal load and therefore provides valuable information that helps to differentiate between infected and colonized patient. This cut off was based on the reference values recovered from the literature as well as in the sensitivity and specificity values obtained in the clinical evaluation of the product. See Section 12. Performance characteristics.**

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

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

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

Table 4. Spectral cross-talk parameters.

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

Step Name	Profile Type	Cycles	Time (s)	Temperature	Detect
Initial denaturation	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection)	2-Temperature	45	10	95°C	-
			41	63°C	✓

Table 5. PCR protocol.

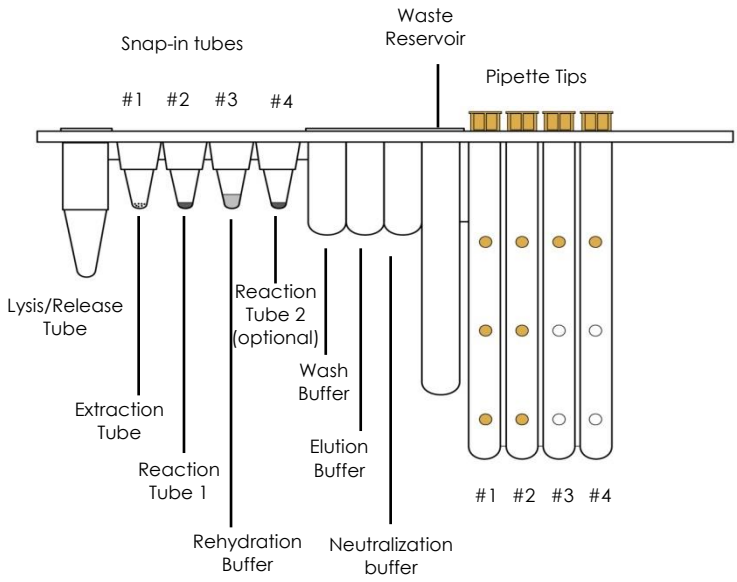
12) Click the "Save Test" button.

8.3.2. BD MAX™ Rack set up

1) For each sample to be tested, remove one Unitized Reagent Strips from the BD MAX™ ExK™ TNA-3 kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes and load on the BD MAX™ System sample racks.
2) Remove the required number of BD MAX™ ExK™ TNA Extraction Tubes (B4) (white foil) from their protective pouch. Snap the Extraction Tube(s) (white foil) into its corresponding positions in the TNA strip (Snap position 1, white color coding on the rack. See Figure 1). Remove excess air, and close pouch with the zip seal.
3) Determine and separate the appropriate number of *Pneumocystis jirovecii* reaction tubes (green or 1D foil) and snap into their corresponding positions in the strip (Snap position 2, green color coding on the rack. See Figure 1).
    a. Remove excess air, and close aluminum pouches with the zip seal.
    b. In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
        i. Note: If you choose the format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Section 8.3.1), determine and separate the appropriate number of additional VIASURE reaction tubes (different foil) and snap into their corresponding positions in the strip (Snap position 4, blue color coding on the rack. See Figure 1). Remove excess air, and close aluminum pouches with the zip seal.

4) Remove the required number of Rehydration Buffer tubes (11 foil) and snap into their corresponding positions in the strip (Snap position 3, non-color coding on the rack. See Figure 1). Remove excess air and close the pouch with the zip seal.
    a. In order to ensure a correct transfer, please make sure that the liquid is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the buffer is at the bottom of the tube.

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



8.3.3. BD MAX™ Instrument set up

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

8.3.4. BD MAX™ report

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

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

9. Result interpretation

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

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

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

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

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

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

Results should be read and analyzed using the following table:

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

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

1 A sample is considered positive if the Ct value obtained is less than 33. The Internal Control (IC) may or may not show an amplification signal. Sometimes, the IC detection is not necessary because a high copy number of the target can cause preferential amplification of target-specific nucleic acids.

2 A sample is considered negative if the sample shows no amplification signal in the detection system, but the internal control is positive (Ct less than 35). An inhibition of the PCR reaction can be excluded by the amplification of internal control. In case of unresolved results (UNR), absence of internal control signal in negative sample it is recommended to repeat the assay following the indications below.

In case of a continued ambiguous result, it is recommended to review the instructions for use, the extraction process used by the user; to verify the correct performance of each PCR steps and review the parameters; and to check the sigmoid shape of the curve and the intensity of fluorescence.

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

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

10. Limitations of the test

- The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.
- Although this assay can be used with other types of samples it has been validated with BAL. In addition, if sputum samples are used, they can be tested with the recommendations cited above.
- For good test performance, the lyophilized product should be at the bottom of the tube and not adhered to the top area of the tube or the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
- An appearance of the reaction mixture in stabilized format, normally found at the bottom of the tube, different from the usual one (without conical shape, inhomogeneous, smaller/larger in size and/or color different from whitish) does not alter the functionality of the test.
- The quality of the test depends on the quality of the sample; proper extracted nucleic acid from respiratory samples must be extracted.
- This test is a qualitative test and does not provide quantitative values or indicate the number of organisms present.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- There is a possibility of false positive results due to cross-contamination by *Pneumocystis jirovecii* suspicious samples containing high concentrations of target DNA or contamination due to PCR products from previous reactions.
- The specific primer and probe combinations for detection of the *mt LSU rRNA* gene used in VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System do not show significant combined homologies with the human genome, human microflora, or other respiratory microorganisms, which might result in predictable false positive.
- False Negative results may arise from several factors and their combinations, including:
    - Improper specimens' collection, transport, storage, and/or handling methods.
    - Improper processing procedures (including DNA extraction).
    - Degradation of the DNA during sample shipping/storage and/or processing.
    - Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown *Pneumocystis jirovecii* strains.
    - Organism levels in the specimen below the limit of detection or cutoff for the assay.
    - The presence of qPCR inhibitors or other types of interfering substances.
    - Failure to follow instructions for use and the assay procedure.

- A positive test result does not necessarily indicate the presence of viable fungus and does not imply that these fungi are infectious or are the causative agents for clinical symptoms. However, a positive result is indicative of the presence of *Pneumocystis jirovecii* targets sequences.
- If diagnostic tests for other respiratory illnesses are negative and the patient's clinical presentation and epidemiological information suggest that *Pneumocystis* infection is possible, then a false negative result should be considered, and a re-testing of the patient should be discussed.
- A negative result does not preclude the presence of *Pneumocystis jirovecii DNA* in a clinical specimen. If clinical observations, patient history and epidemiological information suggest *Pneumocystis* infection, re-testing increasing sample volume should be considered.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *Pneumocystis jirovecii Real* Time PCR Detection Kit for BD MAX™ System, retesting will be required. Unresolved results may be due to the presence of inhibitors in the sample, or an incorrect rehydration of lyophilized reaction mix tube. If there is an instrument failure, Indeterminate or Incomplete results will be obtained.

11. Quality control

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System 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 *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System was tested using clinical samples (bronchoalveolar lavages) already characterized as positive or negative for *P. jirovecii*. The results were as follows:

	Site	Sample type	Workflow	Target
1	Institute of Medical Microbiology and Virology, Technische Universität Dresden (Germany)	Bronchoalveolar lavages	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	*P. jirovecii*

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

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

Site	Comparator assay	Target	TP	TN	FP	FN	Sensitivity	Specificity
1	RealStar® *Pneumocystis jirovecii* PCR assay*	*P. jirovecii*	38	128	0	5	88% (79 – 94)	100% (98 – 100)

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

*** RealStar® *Pneumocystis jirovecii* PCR assay is a qualitative assay, samples with concentrations of ≥3000 copies/ml were considered positive**

Due to the importance of establishing a correct diagnosis, a cut off value was considered in order to obtain an estimation of the fungal burden and therefore distinguish between infected and colonized patient. This cut off was based on the reference values recovered from the literature (1. Louis M, Guitard J, Jodar M, et al. Impact of HIV infection status on interpretation of quantitative PCR for detection of pneumocystis jirovecii. J Clin Microbiol. 2015;53(12):3870-3875; 2. Fauchier T, Hasseine L, Gari-Toussaint M, Casanova V, Marty PM, Pomares C. Detection of pneumocystis jirovecii by quantitative PCR to differentiate colonization and pneumonia in immunocompromised HIV-Positive and HIV-Negative Patients. J Clin Microbiol. 2016;54(6):1487-1495), as well as sensitivity and specificity values obtained in this clinical study. Fungal load higher than $3x10^4$ copies/ml (Ct <30) is very suggestive of *P. jirovecii* Pneumonia, while fungal load below $3x10^3$ copies/ml (Ct>33) usually corresponds to colonization.

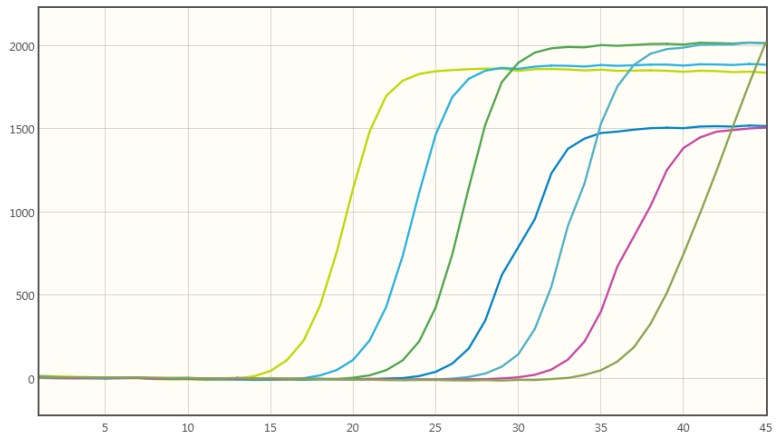
The comparator assay method used in the clinical evaluation was RealStar® Pneumocystis jirovecii PCR (Altona). This method provides quantification of the fungal load since Pneumocystis jirovecii quantification standards are included in each run. From the 43 samples that showed a quantification value >$3x10^3$ copies/ml using the comparator assay, 38 showed Ct value <33 using VIASURE *Pneumocystits jirovecii* Real Time PCR Detection Kit for BDMAX™ System. On the other hand, all the samples that showed a quantification value <$3x10^3$ copies/ml showed a Ct value >33 or were negative.

Result show agreement to detect *Pneumocystis jirovecii* using VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytical sensitivity

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System has a detection limit (LoD) of ≥236 copies per reaction on bronchoalveolar lavages (BALs) with a positive rate of ≥ 95%:

Figure 2. Dilution series of *Pneumocystis jirovecii* ($2.36*10^7$ -$2.36*10^1$ copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).



12.3. Analytical specificity

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

Cross-reactivity testing					
Acinetobacter baumannii	-	HHV6 strain Z29	-	*Legionella dumoffii*	-
Human Adenovirus types 1-5, 8, 15, 31, 40 and 41	-	HHV6 Type A	-	*Legionella longbeachae*	-
Aspergillus fumigatus	-	HHV6 Type B	-	*Legionella micdadei*	-
Bacteroides fragilis	-	HSV-1 strain MacIntyre	-	*Legionella pneumophila*	-
BK Virus Type Ib-2	-	HSV-2 MS	-	*Listeria innocua* Serotype 6a/strain CCUG 15531	-
BK Virus Type IV	-	Influenza A/New Caledonia/20/99(H1N1) virus	-	*Listeria ivanovii* Serovar 5/strain CCUG 15528	-
Bocavirus	-	Influenza A/California/7/2009(H1N1)pdm09-like virus	-	*Listeria monocytogenes* Serotype 1/2b	-
Bordetella bronchiseptica	-	Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-	*Listeria monocytogenes* Serovar 4b/Strain CIP 59.53	-
Bordetella holmesii	-	Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-	Human metapneumovirus A and B	-
Bordetella parapertussis	-	Influenza A/Perth/16/2009(H3N2)-like virus	-	*Moraxella catarrhalis*	-
Bordetella pertussis	-	Influenza A/Thüringen/5/17 (H3N2) virus	-	*Mycoplasma pneumoniae*	-
Candida albicans	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-	Human parainfluenza 1, 2, 3 and 4 viruses	-
Chlamydia caviae	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-	Parvovirus B19	-
Chlamydia psittaci genotype A and C	-	Influenza A/Turkey/Germany R2485+86/2014 (H5N8) virus	-	*Plasmodium falciparum* 3D7	-
Chlamydophila pneumoniae	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8) virus	-	*Pseudomonas aeruginosa*	-
Citomegalovirus strain AD-169	-	Influenza A/Anhui/1/2013 (H7N9) virus	-	Respiratory syncytial virus (RSV)	-
Human coronavirus 229E, OC43 and NL63	-	Influenza B/Brisbane/60/2008-like virus	-	Human rhinovirus	-
MERS Coronavirus	-	Influenza B/Florida/04/06 virus	-	*Staphylococcus aureus* subsp. *aureus*	-
Enterobacter aerogenes Serotype Cloaca B	-	Influenza B/Phuket/3073/2013 virus	-	*Streptococcus pneumoniae*	-
Enterobacter cloacae Serotype Cloaca A	-	JC Virus Type 1A	-	*Toxoplasma gondii* Type II	-
Epstein-Barr virus	-	JC Virus Type 2B	-	*Treponema pallidum*	-
Escherichia coli 0.1285;O18:H7:K1	-	*Klebsiella oxytoca*	-	Varicella-Zoster Virus Ellen	-
Haemophilus influenzae MinnA	-	*Legionella bozemanii*	-	*Yersinia enterocolitica O:3*	-
Hepatitis A	-				

Table 9. Reference pathogenic microorganisms used in this study.

12.4. Analytical reactivity

The reactivity of VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System was evaluated against DNA extracted from *P. jirovecii* Type 1A, *P. jirovecii* g885652 and *P. jirovecii* j888023, showing positive results.

DANSK

1. Anvendelsesformål

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System er en automatiseret realtids PCR test designet til kvalitativ påvisning af *Pneumocystis jirovecii* DNA i respirationsprøver (bronchoalveolar lavaage) fra patienter, der mistænkes for luftvejsinfektion af deres sundhedspersonale (HCP). Denne test er beregnet som en hjælp til identifikation af *Pneumocystis jirovecii* i kombination med patientens kliniske tegn og symptomer og epidemiologiske risikofaktorer. Analysen anvender BD MAX™ System til automatisk ekstraktion af DNA og efterfølgende realtids-PCR med anvendelse af de medfølgende reagenser kombineret med universelle reagenser og engangsartikler til BD MAX™ System. DNA fra respirationsprøver *detekteres* ved hjælp af fluorescerende rapportørfarveprober, der er specifikke for *Pneumocystis jirovecii*.

2. Oversigt og forklaring

Pneumocystis jirovecii pneumoni (PCP) er en akut og livstruende lungesygdom forårsaget af svampen *Pneumocystis jirovecii*. PCP er en vigtig sygdom hos mennesker med immunsvækkende sygdomme, især HIV-smittede, men også patienter med immunforsvar, der er alvorligt nedsat af andre årsager. Hos mennesker med et normalt immunforsvar er det en ekstremt almindeligt forekommende stille infektion. I verdens udviklingsregioner blev forekomsten af PCP engang anset for at være meget lavere, men undersøgelser har vist, at den lavere rapporterede incidens sandsynligvis vil føre til, at der ikke foretages en nøjagtig diagnosticering.

Symptomerne på PCP er ikke-specifikke, og hos HIV-patienter er der en tendens til at sygodmmen optræder meget senere, ofte efter flere ugers symptomer, sammenlignet med PCP, der er forbundet med andre immunkompromitterende forhold. Symptomer på PCP omfatter følgende: Progressiv dyspnø ved anstrengelse, feber, ikke-produktiv hoste, ubehag i brystkassen, vægttab, kuldegysninger og hæmatyse (sjældne).

PCP er vanskelig at diagnosticere som følge af de tilknyttede ikke-specifikke tegn og symptomer. Fordi *P. jirovecii* ikke kan dyrkes i cellekultur, er mikroskopisk visualisering af cyster eller trofiske former i pulmonale prøver med cytokemisk eller immunofluorescensfarvning med monoklonale antistoffer og/eller DNA-forstærkning standardprocedurerne til påvisning af denne mikroorganisme.

3. Procedurens princip

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System er designet til kvalitativ påvisning af DNA fra *Pneumocystis jirovecii* i respirationsprøver. Efter DNA-isolering foretages identifikationen af Pneumocystis jirovecii i ved amplifikation af en bevaret region af den store underenhed af (mt LSU) rRNA-genet ved hjælp af specifikke primere og en fluorescensmærket probe.

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System baseret på 5´exonukleaseaktivitet fra DNA-polymerase. Under DNA-forstærkningen spalter dette enzymproben, som er bundet til den komplementære DNA-sekvens og adskiller quencher-farvestoffet fra rapportøren. Denne reaktion genererer en

stigning i det fluorescerende signal, som er proportional med mængden på målskabelonen. Denne fluorescens måles af BD MAX™ System.

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System indeholder i hvert rør alle de nødvendige komponenter til PCR-analyse (specifikke primere/prober, dNTPS, buffer, polymerase, omvendt transkriptase) i et stabiliseret format samt en intern kontrol til overvågning af ekstraktionsprocessen og/eller hæmning af polymeraseaktiviteten.

Mål	Kanal	Gen
Pneumocystis jirovecii	475/520	Stor underenhed (mt LSU) rRNA-gen
Internal Control (IC)	530/565	-

Tabel 1. Mål, kanal og gener.

4. Leverede reagenser

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System indeholder følgende materialer og reagenser, som er beskrevet i Tabel 2:

Reagens/Materiale	Beskrivelse	Farve/stregkode	Mængde
Pneumocystis jirovecii reaction tube	En blanding af enzymer, primerprober, buffere, dNTP'er, stabilisatorer og interne kontroller i stabiliseret format	Grøn eller 1D folie	2 poser med 12 transparente rør
Rehydration Buffer tube	Opløsning til rekonstitution af det stabiliserede produkt	11 folie	1 pose med 24 transparente rør

Tabel 2. Reagenser og materialer leveret i VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System med Kat. nr. VS-JIR124 (444207).

5. Reagenser og udstyr, der skal leveres af brugeren

Følgende liste omfatter materialer og udstyr, der er nødvendige til brug, men ikke inkluderet VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System.

- Realtids-PCR-instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442828 eller 442827).
- BD MAX™ PCR Cartridges (Ref: 437519).
- vortex.
- Mikropipetter (nøjagtighed mellem 2 og 1000 µl).
- N-acetyl-L-cystein (anbefalet N-acetyl-L-cystein ref. A7250, Merck KGaA).
- Nukleasefrit vand.
- Filterspidser.
- Pulverfrie engangshandsker.

6. Transport- og opbevaringsforhold

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

7. Særlige forholdsregler for brugere

- Produktet er kun beregnet til brug af professionelle brugere, f.eks. laboratorie- eller sundhedspersonale og teknikere, der er uddannet i molekylærbiologiske teknikker.
- Til *in vitro*-diagnostisk brug.
- Brug ikke reagenser og/eller materialer, hvis udløbsdatoen er overskredet.
- Brug ikke sættet, hvis etiketten, der forsegler den ydre æske, er i stykker.
- Brug ikke reagenser, hvis beskyttelsesæsken er åben eller i stykker ved ankomsten.
- Brug ikke reagenser, hvis beskyttelsesposerne er åbne eller i stykker ved modtagelsen.
- Brug ikke reagenser, hvis tørremidlet ikke er til stede eller er i stykker inden i reagensposerne.
- Tørremidlet må ikke fjernes fra reagensposerne.
- Luk straks de beskyttende poser med reagenser med lynlåsforseglingen efter hver brug. Fjern eventuel overskydende luft i poserne inden forsegling.
- Brug ikke reagenser, hvis folien er blevet ødelagt eller beskadiget.
- Reagenser fra forskellige poser og/eller sæt og/eller partier må ikke blandes.
- Beskyt reagenser mod fugt. Længerevarende eksponering for fugt kan påvirke produktets ydeevne.
- Hold komponenterne væk fra lys.
- I tilfælde, hvor andre PCR-test udføres i det samme generelle område af laboratoriet, skal det sikres, at VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3-ekstraktionssættet, eventuelle yderligere reagenser, der er nødvendige for testen, og BD MAX™ System ikke er kontamineret. Undgå altid mikrobiel og ribonuklease (RNase) / deoxyribonuklease (DNase) kontaminering af reagenser. Det anbefales at anvende sterile RNase/DNase-fri aerosolresistente engangspipettespidser eller positive fortrængningspipettespidser. Brug en ny spids til hver prøve. Handsker skal udskiftes før håndtering af reagenser og kassetter (BD MAX™ PCR Cartridge).
- For at undgå kontaminering af miljøet med amplikoner må BD MAX™ PCR Cartridge ikke brydes fra hinanden efter brug. Forseglingerne på BD MAX™ PCR Cartridge er designet til at forhindre kontaminering.
- Tilrettelæg en ensrettet arbejdsgang. Den skal begynde i ekstraktionsområdet og derefter flyttes til forstærknings- og detektionsområdet. Prøver, udstyr og reagenser må ikke returneres til det område, hvor det foregående trin blev udført.
- Følg god laboratoriepraksis. Brug beskyttelsestøj, engangshandsker, beskyttelsesbriller og maske. Man må ikke spise, drikke, ryge eller lægge makeup i arbejdsområdet. Vask hænder efter endt test.
- Prøverne skal behandles som potentielt smitsomme og/eller biologisk farlige, samt alle reagenser og materialer, der er blevet eksponeret for prøverne, og skal håndteres i overensstemmelse med de nationale sikkerhedsforskrifter. Træf de nødvendige forholdsregler under indsamling, opbevaring, behandling og bortskaffelse af prøver.
- Prøver og reagenser skal håndteres i et biologisk sikkerhedsskab. Anvend personlige værnemidler (PPE) i overensstemmelse med gældende retningslinjer for håndtering af potentielt smitsomme prøver. Affald bortskaffes i overensstemmelse med lokale retningslinjer.
- Regelmæssig dekontaminering af almindeligt anvendt udstyr anbefales, især mikropipetter og arbejdsflader.
- "I overensstemmelse med Forordning (EF) nr. 1907/2006 (REACH), kræver VIASURE Real Time PCR Detection Kits ikke materialesikkerhedsdatablade (Material Safety Data Sheets) som en del af deres klassificering som værende ufarlige for helbredet og miljøet, fordi de ikke indeholder stoffer og/eller blandinger, som opfylder

kriterierne for fareklassificering iht. forordning (EF) nr. 1272/2008 (CLP), eller forefindes i koncentrationer, der er højere end den værdi, der er angivet i den nævnte forordning til deres erklæring.

- Se brugervejledningen til BD MAX™ System for yderligere advarsler, forholdsregler og procedurer.

8. Analysemetode

8.1. Prøveindsamling, opbevaring og transport

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System er testet på bronchoalveolar lavoles (BALs). Andre typer prøver skal valideres af brugeren.

Prøveudtagning, opbevaring og transport skal vedligeholdes i overensstemmelse med de betingelser, der er valideret af brugeren. Samlet set skal luftvejsprøver indsamles og mærkes på passende vis i rene beholdere med eller uden transportmidler (afhængigt af prøvetype) og behandles så hurtigt som muligt for at garantere testens kvalitet. Prøverne skal transporteres ved 4 °C i op til 7 dage i henhold til lokale og nationale bestemmelser for transport af patogent materiale. Ved langtidstransport (mere end 7 dage) anbefaler vi forsendelse ved ≤-20 °C eller lavere. Det anbefales at anvende friske prøver til testen. Prøverne kan opbevares ved 4 °C i op til 7 dage eller nedfryses ved -20 °C eller ideelt ved -80 °C for konservering. Gentagne fryse-tø-cyklusser bør undgås for at forhindre nedbrydning af prøven og nukleinsyrer.

Respirationsprøverne skal indsamles, transporteres og opbevares i overensstemmelse med relevante laboratorieretningslinjer. For yderligere oplysninger henvises til CDC guideline (CDC-retningslinjer for prøveudtagning. Websted https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf) og IDSA-retningslinjerne (Miller, J. M., Binnicker, M. J., Campbell, S.,... & Pritt, B. S. (2018). En vejledning i anvendelse af mikrobiologilaboratoriet til diagnosticering af smitsomme sygdomme: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Kliniske infektionssygdomme*, 67(6), e1-e94).

Hvis der anvendes sputumprøver, kan de testes i henhold til nedenstående anbefalinger.

8.2. Prøveklargøring og DNA-ekstraktion

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

1. Der pipetteres 200 BAL over i et BD MAX™ ExK™ TNA-3 Sample Buffer Tube, og røret lukkes med en septumhætte. Der sikres fuldstændig blanding ved at vortexe prøven ved høj hastighed i 1 minut. Fortsæt til BD MAX™ System Operation betjening.

2. Til sputumprøver tilsættes acetylcystein (anbefalet N-acetyl-L-cystein ref. A7250, Merck KGaA) til prøven i forholdet 1:1 (dvs. 250 µl sputum og 250 µl acetylcystein 100 mg/ml), blandes ved omrystning og opvarmes til 95 °C i 10 minutter. Der pipetteres 200 µl BD MAX™ ExK™ TNA-3 Sample Buffer Tube over i et BD MAX™ TNA-3 Sample Buffer Tube, og røret lukkes med en septumhætte. Der sikres fuldstændig blanding ved at vortexe prøven ved høj hastighed i 1 minut. Fortsæt til BD MAX™ System Operation betjening.

8.3. PCR-protokol

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

8.3.1. Oprettelse af PCR-testprogram til VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System

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

1) Vælg fanen "Test Editor" (Testredigering) på skærmen "Run" (Kør) på BD MAX™ System.
2) Klik på knappen "Create" (Opret).
3) Navngiv din test i fanen Basic Information (Grundlæggende oplysninger) i vinduet "Test Name" (Testnavn): dvs. VIASURE *Pneumocystis jirovecii*.
4) I rullemenuen "Extraction Type" (Ekstraktionstype), vælg "ExK TNA-3".
5) Vælg "Type 5" i rullemenuen "Master Mix Format".
    a. Bemærk: Produktet kan anvendes i kombination med en ekstra VIASURE til BD MAX test, og vælg derefter "Dual Master Mix Concentrated Lyofized MM with Rehydration Buffer (Type 5)".
6) I "Sample extraction parameters" (Parametre for prøveekstraktion) vælges "User defined" (Brugerdefineret), og prøvevolumen justeres til 700 µl.
7) I "Ct Calculation" (Ct-beregning) vælges "Call Ct at Threshold Crossing" (Beregn Ct når tærsklen krydses).
8) Hvis du kører softwareversion 5.00 eller nyere og har snap-in-rør med stregkodet folie, skal du vælge følgende konfiguration i "Custom Barcodes" (Brugerdefinerede stregkoder):
    a. Snap-In 2 Barcode (Snap-In 2-stregkode): 1D (vedrørende *Pneumocystis jirovecii* reaction tube).
    b. Snap-In 3 Barcode (Snap-In 3-stregkode): 11 (vedrørende Rehydration Buffer tube).
    c. Snap-In 4 Barcode (Snap-In 4-stregkode): et andet VIASURE reaction tube (forskellig folie), hvis du vælger formatet "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Afsnit 8.3.1).
9) Indtast følgende parametre på fanen "PCR settings" (PCR-indstillinger): "Channel Settings" (Kanalindstillinger), "Gains" (Stigninger) og "Threshold" (Tærskel) (Tabel 3).
    a. Bemærk: Produktet kan anvendes i kombination med en ekstra VIASURE til BD MAX-test, PCR-indstillinger og testtrin skal gennemføres for position 2 (grøn) og position 4 (blå).

Channel (Kanal)	Alias (Alias)	Gain (Gevinst)	Threshold (Tærskel)	Ct Min (Ct Min)	Ct Max (Ct Max)
475/520 (FAM)	*P. jirovecii*	50	200	0	33*
530/565 (HEX)	IC	80	200	0	35
585/630 (ROX)	-	0	0	0	0
630/665 (Cy5)	-	0	0	0	0
680/715 (Cy5.5)	-	0	0	0	0

Tabel 3. "PCR settings" (PCR-indstillinger).

* Brugen af en klinisk tærskel på Ct 33 i dette testsystem (svarende til 3000 kopier/ml) gør det muligt at skelne mellem høj og lav fungal belastning og giver derfor værdifuld information, der hjælper med at skelne mellem inficeret og koloniseret patient. Denne afskæring var baseret på referenceværdierne fra litteraturen samt på de sensitivitets- og specificitetsværdier, der blev opnået ved den kliniske evaluering af produktet. Se Punkt 12. Ydelseskarakteristika.

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

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

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

Tabel 4. Parametre for "Spectral Cross Talk" (spektral krydstale).

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

Step Name (Trinnavn)	Profile Type (Profiltype)	Cycles (Cyklusser)	Time (s) (Tid(er))	Temperature (Temperatur)	Detect (Registrering)
Initial denaturation (Indledende denaturering)	Hold	1	120	98 °C	-
Denaturation and Annealing/Extension (Data collection) (Denaturering og annotering/udvidelse (dataindsamling))	2-temperatur	45	10	95 °C	-
			41	63 °C	✓

Tabel 5. PCR-protokol.

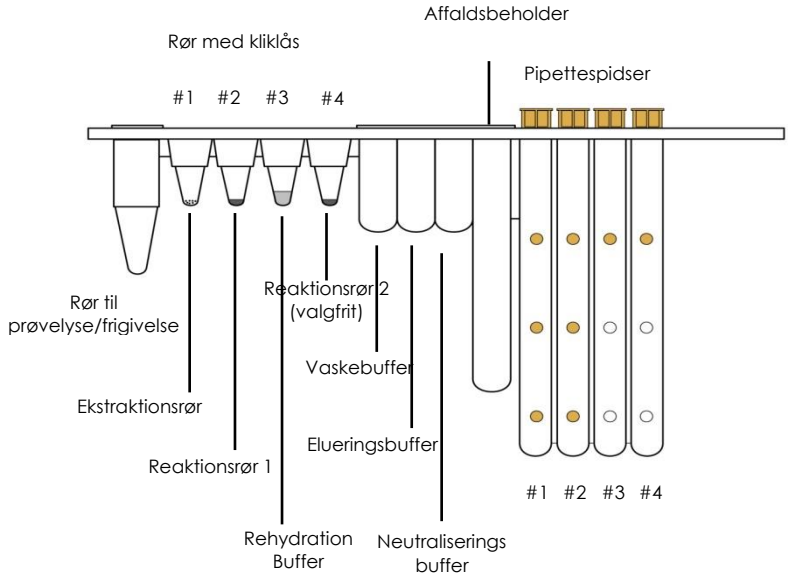
12) Klik på knappen "Save Test" (Gem test).

8.3.2. Opsætning af BD MAX™-stativ

1) For hver prøve, der skal testes, fjernes en Unitized Reagent Strips (samlet reagensstrimmel) fra BD MAX™ ExK™ TNA-3 kit. Bank forsigtigt hver strimmel mod en hård overflade for at sikre, at alle væskerne ligger i bunden af rørene, og anbring dem i BD MAX™ Systems prøvestativer.
2) Fjern det nødvendige antal BD MAX™ ExK™ TNA Extraction Tubes (B4) (hvid folie) fra deres beskyttelsespose. Sæt udtræksrøret(-rørene) (hvid folie) i de tilsvarende positioner i TNA-strimlen (fastgør position 1, hvid farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk posen med lynlåsforseglingen.

3) Identificer og adskil det rette antal *Pneumocystis jirovecii* reaction tubes (reaktionsrør) (grøn eller 1D folie), og klik dem på plads i deres tilsvarende positioner på strimmel (klik-position 2, grøn farvekodning på stativet. Se Figur 1).
    a. Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    b. Rehydreringen udføres korrekt ved at sørge for, at det frysetørrede produkt ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.
        ii. Bemærk: Hvis du vælger formatet "Dual Master Mix Concentrated Lyophilised MM with Rehydration Buffer (Type 5)" (Dobbelt mastermix koncentreret frysetørret MM med rehydreringsbuffer (type 5)) (afsnit 8.3.1), bestemmes og adskilles det passende antal ekstra VIASURE reaktionsrør (forskellig folie) og klikkes fast i deres tilsvarende positioner i strimlen (klik-position 4, blå farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    c. Fjern det nødvendige antal Rehydration Buffer tubes (11 folie), og klik dem fast på deres tilsvarende pladser på strimlen (klik-position 3, ikke-farvet kodning på stativet. Se Figur 1). Fjern overskydende luft, og luk posen med lynlåsforseglingen.
        i. For at sikre, at overførslen udføres korrekt, skal man sørge for, at væsken ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt på hvert rør på en hård overflade for at sikre, at al bufferen er i bunden af røret.

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



8.3.3. BD MAX™ Instrumentopsætning

1) Vælg fanen "Work List" (Arbejdsliste) på skærmen "Run" (Kør) på BD MAX™ Systemsoftware v4.50A eller nyere.
2) Vælg VIASURE *Pneumocystis jirovecii* i rullemenuen "Test" (hvis det ikke allerede er oprettet, se afsnit 8.3.1).
3) Vælg det relevante lotnummer for kittet (fremgår af ekstraktionskittets udvendige æske) fra rullemenuen (valgfrit).
4) Indtast prøvebufferrørets identifikationsnummer i vinduet Sample tube (Prøverør) på Worklist (Arbejdsliste), enten ved at scanne stregkoden med scanneren eller ved manuel indtastning.

5) Udfyld prøven/patient-id'et og/eller adgangsvinduet på arbejdslisten, og klik på knappen "Save" (Gem). Fortsæt, indtil alle Sample Buffer Tubes (prøvebufferrør) er indtastet. Sørg for, at prøve-/patient-id'et og Sample Buffer Tubes matcher nøjagtigt.
6) Anbring det klargjorte Sample Buffer Tube i BD MAX™ Rack(s) (stativet/stativerne).
7) Sæt stativet/stativerne i BD MAX™ System (stativ A er placeret i venstre side af BD MAX™ System og stativ B i højre side).
8) Anbring det nødvendige antal BD MAX™ PCR Cartridges i BD MAX™ System.
9) Luk lågen til BD MAX™ System.
10) Klik på "Start Run" (Start procedure) for at starte proceduren.

8.3.4. BD MAX™ rapport

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

9. Tolkning af resultater

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

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

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

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

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

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

Resultaterne skal læses og analyseres ved hjælp af følgende tabel:

Pneumocystis jirovecii (475/520)	Intern kontrol (530/565)	Fortolkning
+	+/-[1]	***Pneumocystis jirovecii* Detekteret 1**
-	+/-[1]	***Pneumocystis jirovecii* Ikke detekteret [1]**
-	-[2]	**Resultatet Unresolved (uløst) (UNR) optræder under tilstedeværelse af hæmmere i PCR-reaktionen eller når der opstår et overordnet problem (der ikke rapporteres med en fejlkode) under prøvekørslen og/eller forstærkningstrinnene.[2]**
IND	IND	**Analyseresultatet er Indeterminate (ubestemmeligt) (IND). Skyldes en fejl i BD MAX™ System. Analyseresultat, der vises i tilfælde af en instrumentfejl, der knyttet til en fejlkode.**
INC	INC	**Analyseresultatet er Incomplete (ufuldstændigt) (INC). Skyldes en fejl i BD MAX™ System. Analyseresultatet vises, hvor en fuldstændig kørsel ikke kunne gennemføres.**

Tabel 6. Prøvefortolkning.
+: Der opstod forstærkning.
-: Der opstod ingen forstærkning.

1 En prøve betragtes som positiv, hvis Ct-værdien er mindre end 33. Den interne kontrol (IC) kan både vise et forstærkersignal eller intet forstærkersignal. Sommetider er IC-detektionen ikke nødvendig, fordi et højt kopinummer for målet kan forårsage præferenceamplifikation af målspecifikke nukleinsyrer.

2 En prøve betragtes som negativ, hvis prøven viser intet forstærkningssignal, men den interne kontrol er positiv (Ct mindre end 35). En hæmning af PCR-reaktioner kan udelukkes ved forstærkningen af intern kontrol. I tilfælde af uløste resultater (UNR), manglende internt kontrolsignal i en negativ prøve anbefales det at gentage analysen ved følgende indikationer angivet.

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

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

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

10. Begrænsninger i testen

- Resultaterne af testen bør vurderes af en læge på baggrund af anamnese, kliniske symptomer og andre diagnostiske tests.
- Selv om denne test kan bruges sammen med andre typer prøver er den blevet valideret med indsamlet i BAL. Hvis der anvendes sputumprøver, kan de testes i henhold til ovennævnte anbefalinger.
- For god testydeevne skal det frysetørrede produkt være i bunden af røret og ikke klæbe til det øverste område af røret eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.

- Et udseende af reaktionsblandingen i stabiliseret format, som normalt findes i bunden af røret, forskelligt fra det sædvanlige (uden konisk form, inhomogent, mindre/større i størrelse og/eller farve forskellig fra hvidlig) ændrer ikke testens funktionalitet.
- Testens kvalitet afhænger af prøvens kvalitet; korrekt ekstraheret nukleinsyre fra luftvejsprøver skal ekstraheres.
- Denne test er en kvalitativ test og giver ikke kvantitative værdier eller angiver antallet af tilstedeværende organismer.
- Meget lave målniveauer under detektionsgrænsen kan påvises, men resultaterne er muligvis ikke reproducerbare.
- Der er mulighed for falske-positive resultater som følge af krydskontaminering af *Pneumocystis jirovecii*-mistænkte prøver, der indeholder høje koncentrationer af mål-DNA eller er kontaminerede med PCR-produkter fra tidligere reaktioner.
- De specifikke primer- og probekombinationer til påvisning af *mt LSU rRNA genet*, der anvendes i VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System, viser ikke signifikante kombinerede homologier med det humane genom, menneskelig mikroflora eller andre coronavirus, som kan resultere i forudsigelig falsk positiv.
- Falsk-negative resultater kan skyldes flere faktorer og kombinationer heraf, herunder:
    - Forkerte metoder til indsamling, transport, opbevaring og/eller håndtering af prøver.
    - Forkerte behandlingsprocedurer (herunder DNA-ekstraktion).
    - Nedbrydning af DNA under forsendelse/opbevaring og/eller behandling af prøver.
    - Mutationer eller polymorfismer i primer- eller probebindingsområder kan påvirke påvisningen af nye eller ukendte *Pneumocystis jirovecii*-varianter.
    - Organismemængde i prøven under detektionsgrænsen for analysen.
    - Tilstedeværelsen af qPCR-hæmmere eller andre typer interfererende stoffer.
    - Manglende overholdelse af brugsanvisningen og analyseproceduren.
- Et positivt testresultat indikerer ikke nødvendigvis tilstedeværelsen af levedygtige vira og betyder ikke, at disse vira er smitsomme, eller de forårsager kliniske symptomer. Et positivt resultat indikerer imidlertid tilstedeværelsen af *Pneumocystis jirovecii*-målvirussekvenser.
- Hvis diagnostiske test for andre luftvejssygdomme er negative, og patientens kliniske præsentation og epidemiologiske oplysninger antyder, at *Pneumocystis*-infektion er mulig, bør et falsk negativt resultat overvejes, og en ny test af patienten bør drøftes.
- Et negativt resultat udelukker ikke tilstedeværelsen af *Pneumocystis jirovecii DNA* i en klinisk prøve. Hvis kliniske observationer, patientanamnesen og epidemiologiske oplysninger antyder infektion med *Pneumocystis jirovecii*, bør det overvejes at gentage testen med en øget prøvevolumen.
- Hvis der opnås uafklarede, ubestemte eller ufuldstændige resultater ved hjælp af VIASURE *Pneumocystis jirovecii Real* Time PCR Detection Kit for BD MAX™ System, kræves en ny test. Uløste resultater kan skyldes tilstedeværelsen af hæmmere i prøven eller forkert rehydrering af frysetørrede reaktionsblandingsrør. Hvis der opstår en instrumentfejl, kan det medføre ubestemmelige eller ufuldstændige resultater.

11. Kvalitetskontrol

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System indeholder en intern kontrol (IC) i hvert reaktionsrør, der bekræfter korrekt teknikydelse.

12. Ydelseskarakteristika

12.1. Klinisk sensitivitet og specificitet

Den kliniske ydeevne af VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System blev testet ved hjælp af kliniske prøver (nasopharyngeale podepinde), der allerede var karakteriseret som positive eller negative for *P. jirovecii* fra patienter. Resultaterne var følgende:

	Center	Prøvetype	Arbejdsgang	Mål
1	Institut for medicinsk mikrobiologi og Virologi, Technische Universität Dresden (Tyskland)	Bronchoalveolar lavage	BD MAX™ ExK™ TNA-3 kit + BD MAX™ System	*P. jirovecii*

Tabel 7. Sted, prøvetype, arbejdsgang og mål.

Sand-positive og -negative værdier, falsk-positive og -negative værdier, følsomhed, specificitetsværdier for VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System blev beregnet i forhold til hver komparatoranalyse som vist i følgende tabel:

Center	Komparatoranalyse	Mål	TP	TN	FP	FN	Følsomhed	Specificitet
1	RealStar® *Pneumocystis jirovecii* PCR assay*	*P. jirovecii*	38	128	0	5	88% (79 – 94)	100% (98 – 100)

Tabel 8. Sand-positive (TP) eller -negative (TN) værdier, falsk-positive (FP) og -negative (FN) værdier, sensitivitets-, specificitetværdier for VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System.

*** RealStar Pneumocystis jirovecii PCR-assay er en kvalitativ analyse, hvor prøver med koncentrationer på ≥ 3000 kopier/ml blev betragtet som positive**

På grund af vigtigheden af at etablere en korrekt diagnose, blev der taget hensyn til en skæringsværdi for at opnå et skøn over den fungale belastning og således skelne mellem en inficeret og koloniseret patient. Dette skæringspunkt var baseret på referenceværdierne fra litteraturen (1. Louis M, Guitard J, Jodar M, et al. Impact of HIV infection status on interpretation of quantitative PCR for detection of pneumocystis jirovecii. J Clin Microbiol. 2015;53(12):3870-3875; 2. Fauchier T, Hasseine L, Gari-Toussaint M, Casanova V, Marty PM, Pomares C. Detection of pneumocystis jirovecii by quantitative PCR to differentiate colonization and pneumonia in immunocompromised HIV-Positive and HIV-Negative Patients. J Clin Microbiol. 2016;54(6):1487-1495), samt sensitivitets- og specificitetsværdier, der blev indhentet i forbindelse med dette kliniske forsøg. En fungal belastning højere end $3x10^4$ kopier/ml (CT<30) er en tydelig indikator for *P. jirovecii* pneumoni, mens en fungal belastning på under $3x10^3$ kopier/ml (CT>33) normalt svarer til kolonisering.

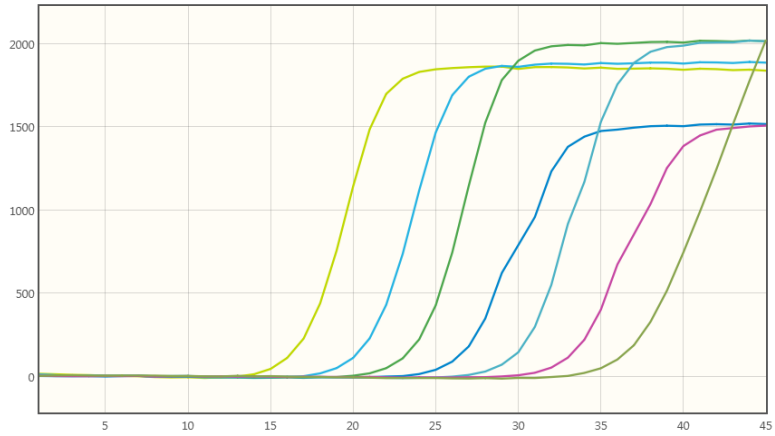
Den sammenligningsanalysemetode, der blev anvendt i den kliniske evaluering, var RealStar Pneumocystis jirovecii PCR (Altona). Denne metode giver kvantificering af fungal belastning, da Pneumocystis jirovecii kvantificeringsstandarder er inkluderet i hver kørsel. Fra de 43 prøver, der viste en kvantificeringsværdi > $3x10^3$ kopier/ml ved hjælp af sammenligningsanalysen, viste 38 CT-værdi <33 ved brug af VIASURE *Pneumocystits jirovecii* Real Time PCR Detection Kit for BDMAX™ System. På den anden side viste alle prøver, der viste en kvantificeringsværdi <3x103 kopier/ml, en CT-værdi > 33 eller var negative.

Resultaterne viser høj ensartethed ved detektering af *Pneumocystis jirovecii*i ved hjælp af VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytisk sensitivitet

VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse (LoD) på ≥ 236 kopier pr. reaktion på bronchoalveolar lavninger (BALs) med en positiv hastighed på ≥ 95 %:

*Figur 2. Fortyndingsserie til Pneumocystis jirovecii (2,36*107-2,36*101 kopier pr. reaktion) skabelon kørt på BD MAX™ System (kanalen 475/520 (FAM)).*



12.3. Analytisk specificitet

Specificiteten af *Pneumocystis jirovecii*-analysen blev bekræftet ved at teste et panel bestående af forskellige mikroorganismer, der repræsenterer de mest almindelige respiratoriske patogener. Der blev ikke påvist krydsreaktivitet mellem nogen af følgende testede mikroorganismer:

Krydsreaktivitetsanalyse					
Acinetobacter baumannii	-	HHV6 strain Z29	-	*Legionella dumoffii*	-
Human Adenovirus-type 1-5, 8, 15, 31, 40 og 41	-	HHV6 Type A	-	*Legionella longbeachae*	-
Aspergillus fumigatus	-	HHV6 Type B	-	*Legionella micdadei*	-
Bacteroides fragilis	-	HSV-1 strain MacIntyre	-	*Legionella pneumophila*	-
BK Virus Type Ib-2	-	HSV-2 MS	-	*Listeria innocua* Serotype 6a/strain CCUG 15531	-
BK Virus Type IV	-	Influenza A/New Caledonia/20/99(H1N1)-virus	-	*Listeria ivanovii* Serovar 5/strain CCUG 15528	-
Bocavirus	-	Influenza A/California/7/2009(H1N1)pdm09-lignende virus	-	*Listeria monocytogenes* Serotype 1/2b	-
Bordetella bronchiseptica	-	Influenza A/Michigan/45/2015 (H1N1)pdm09-virus	-	*Listeria monocytogenes* Serovar 4b/Strain CIP 59.53	-
Bordetella holmesii	-	Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09-virus	-	Menneskelig t metapneumovirus A og B	-
Bordetella parapertussis	-	Influenza A/Perth/16/2009(H3N2)-lignende virus	-	*Moraxella catarrhalis*	-
Bordetella pertussis	-	Influenza A/Thüringen/5/17 (H3N2)-virus	-	*Mycoplasma pneumoniae*	-
Candida albicans	-	Influenza A/Switzerland/9715293/2013 (H3N2)-virus	-	Menneskelig parainfluenza 1, 2, 3 og 4 vira	-

Krydsreaktivitetsanalyse					
Chlamydia caviae	-	Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2)-virus	-	Parvovirus B19	-
Chlamydia psittaci genotype A og C	-	Influenza A/Turkey/Germany R2485+86/2014 (H5N8) virus	-	*Plasmodium falciparum* 3D7	-
Chlamydophila pneumoniae	-	Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8)-virus	-	*Pseudomonas aeruginosa*	-
Citomegalovirus strain AD-169	-	Influenza A/Anhui/1/2013 (H7N9)-virus	-	Respiratorisk syncytial virus (RSV)	-
Human coronavirus 229E, OC43 og NL63	-	Influenza B/Brisbane/60/2008-lignende virus	-	Menneskelig rhinovirus	-
MERS Coronavirus	-	Influenza B/Florida/04/06-virus	-	*Staphylococcus aureus* subsp. *aureus*	-
Enterobacter aerogenes Serotype Cloaca B	-	Influenza B/Phuket/3073/2013 virus	-	*Streptococcus pneumoniae*	-
Enterobacter cloacae Serotype Cloaca A	-	JC Virus Type 1A	-	*Toxoplasma gondii* Type II	-
Epstein-Barr virus	-	JC Virus Type 2B	-	*Treponema pallidum*	-
Escherichia coli 0.1285;O18:H7:K1	-	*Klebsiella oxytoca*	-	Varicella-Zoster Virus Ellen	-
Haemophilus influenzae MinnA	-	*Legionella bozemanii*	-	*Yersinia enterocolitica O:3*	-
Hepatitis A	-				

Tabel 9. Referencepatogene mikroorganismer, der blev brugt i denne undersøgelse.

12.4. Analytisk reaktivitet

Reaktiviteten af VIASURE *Pneumocystis jirovecii* Real Time PCR Detection Kit for BD MAX™ System blev evalueret mod DNA udvundet fra P. jirovecii Type 1A , P. jirovecii g885652 og P. jirovecii j888023, der viste positive resultater.

Bibliography/ Bibliografi

1. A. Roux et al. Pneumocystis jirovecii pneumonia in patients with or without AIDS, France. Emerging Infectious Diseases journal 2014; 20: 1490–1497.
2. E.J. Calderón et al. Pneumocystis infection in humans: diagnosis and treatment. Expert Review of Anti-infective Therapy 2010; 8: 683–701.
3. J.R. Harris et al. Pneumocystis Jirovecii Pneumonia: Current Knowledge and Outstanding Public Health Issues. Current Fungal Infection Reports Journal, 2010; 4(4): 229-237.
4. P. Rohner et al. Detection of Pneumocystis jirovecii by two staining methods and two quantitative PCR assays. Infection, 2009; 37(3):261-5.

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

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

Trademarks

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Change Control / Ændringskontrol		
Version No. / Versionsnr.	**Changes / Ændringer**	**Date / Dato**
00	Original version / Original version.	03/02/2022
01	Typo corrections / Trykfejl rettelser	08/03/2022

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

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