DATA SHEET

Pneumocystis jirovecii (carinii) Real-TM Real Time Kit

for the detection of *Pneumocystis jirovecii (carinii)* in biological materials

NAME

Pneumocystis jirovecii (carinii) Real – TM

INTENDED USE

The **Pneumocystis jirovecii (carinii) Real-TM** is a Real-Time test for the qualitative detection of *Pneumocystis jirovecii (carinii)* in biological materials. DNA is extracted from samples, amplified using real time amplification with fluorescent reporter dye probes specific for *Pneumocystis jirovecii (carinii)* and Internal Control (IC). Test contains an IC (β-globine gene) which serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

MATERIALS PROVIDED

- **PCR-mix-1 Pneumocystis jirovecii (carinii)/Glob,** 0,42 ml
- **PCR-buffer-FRT**,2 x 0,3 ml
- **TaqF Polymerase,** 2 x 0,02 ml
- **Positive Control *Pneumocystis jirovecii (carinii)* and Human DNA C+,** 0,1 ml
- **Negative Control C-**, 1,2 ml*
- **DNA-buffer**, 0,5 ml

Contains reagents for 55 tests.
* *must be used in the isolation procedure as Negative Control of Extraction.*

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA isolation kit (see DNA isolation)
- Desktop microcentrifuge for “eppendorf” type tubes
- Vortex mixer
- Disposable gloves, powderless

Gentaur Molecular Products
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1910 Kampenhout, Belgium

- Biohazard waste container
- Refrigerator
- Freezer
- Real Time Thermal cycler
- Reaction tubes or plate
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Tube racks

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.

2. Do not pipette by mouth.

3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.

4. Do not use a kit after its expiration date.

5. Dispose of all specimens and unused reagents in accordance with local regulations.

6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.

7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.

8. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.

9. Material Safety Data Sheets (MSDS) are available on request.

10. Use of this product should be limited to personnel trained in the techniques of amplification.

11. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

Pneumocystis jirovecii (carinii) Real-TM must be stored – 20°C. The kit can be shipped at 2-8°C for 3-4 days but should be immediately stored at -20°C on receipt.

STABILITY

Pneumocystis jirovecii (carinii) Real-TM is stable up to the expiration date indicated on the kit label.The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Pneumocystis jirovecii (carinii) Real-TM can analyze DNA extracted with **DNA-Sorb-B** from:

• *Sputum, bronchial or tracheal lavage* must be treated with the following procedure*:

1. Prepare the NALC solution for 5 samples: add in the sterile container 250 mg of N-acetyl-L-cysteine, 25 ml 4% NaOH, 25 ml 2.94% sodium citrate.
2. In a biological safety cabinet, using a sterile 50 ml tube, add 1 volume of specimen to 1 volumes of NALC solution (approx. 10 ml of each), vortex for 15-20 sec and incubate 15 min at room temperature with occasional gently shaking.
3. Wash the digested-decontaminated specimens by adding of 0,067M Phosphate buffer (pH 6,8) to the 50 ml mark on the centrifuge tube and mix by inverting. Centrifuge for 15 min at 3000g.
4. Carefully discard the supernatant and leave about 100 µl of solution. Resuspend the sediment. Use the suspension for the DNA extraction.

• *bronchial lavage:* centrifuge 10 mL at 7000 g/min for 10-15 min. Remove and discard the supernatant. If the pellet isn't visible add 10 ml of liquid and repeat centrifugation remove and discard the supernatant. Resuspend the pellet in 100 µl of saline water.

• *tissue* (~1,0 gr) homogenized with mechanical homogenizer or scalpel, glass sticks, teflon pestles and dissolved in 1,0 ml of saline water or PBS sterile (1 volume of tissue to 1 volumes of saline solution). Vortex vigorously and incubate 30 min at room temperature. Transfer the supernatant into a new 1,5 ml tube;

• *swabs:* insert the swab into the nuclease-free 1,5 ml tube and add 0,2 mL of Transport medium. Vigorously agitate swabs in medium for 15-20 sec. It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

The following kit is recommended: **DNA-Sorb-B** Please carry out DNA extraction according to the manufacture's instruction.

PROTOCOL (Reaction volume 25 µl):

1. Prepare Mix of PCR-buffer-FRT and TaqF Polymerase by adding **20 µl** of **TaqF Polymerase** in the tube with **PCR-buffer-FRT**. This mix is stable for 3 months at + 2-8°C. If you don't need to use the all volume in 3 months, it is possible to prepare a lower quantity of Mix of PCR-buffer-FRT and TaqF Polymerase at the rate of 15 to 1, (for example **150 µl** of **PCR-buffer-FRT** and **10 µl** of **TaqF Polymerase).**

2. Prepare required quantity of reaction tubes for samples and controls and add for each tube **7 µl** of **PCR-mix-1** and **8 µl** of **Mix** (PCR-buffer-FRT with TaqF Polymerase).

3. Add **10 µl** of **extracted DNA** sample to appropriate tube.

(*Re-centrifuge all the tubes with extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. N.B. don't disturb the pellet, sorbent inhibit reaction!).*

4. Prepare for each panel 2 controls:

- add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
- add **10 µl** of ***Pneumocystis jirovecii (carinii)*** **and Human DNA C+** to the tube labeled. *C+*;

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line.

β-globine gene (IC) is detected on the FAM (Green) channel, *Pneumocystis jirovecii (carinii)* on the JOE (Yellow)/HEX/Cy3 channel.

The analytical sensitivity of the kit **Pneumocystis jirovecii (carinii) Real-TM** is 200 copies/ml

Real Time Amplification with Rotor-Gene 2000/3000/6000

1. Close tubes and transfer them into the Rotor-Gene 2000/3000/6000.
2. Click *New* in the main menu, select *Dual Labeled Probe.* Click *New*
3. Select Rotor Type *36-Well Rotor* and *No Domed 0,2 ml Tubes.*
4. Reaction volume, 25 µl
5. Program Rotor-Gene 2000/3000/6000 as follows:

1. Hold 95 °C - 15 min
2. Cycling 95 °C - 15 s
60 °C - 45 s - detection
Cycle repeats – 45 times.
*fluorescence detection on the channels Fam (Green), Joe (Yellow) on the 2-nd pass (60°C)

Make the adjustment of the fluorescence channel sensitivity: *Channel Setup →Calibrate (Gain Optimisation for RG6000) → Perform Calibration (Optimisation) Before 1-st Acquisition.* In the window *"Channel Settings"* indicate *Min Reading* 3, *Max Reading* 8 for the channels Fam (Green) and Joe (Yellow). In the column *Tube position* program position of the tubes in the carousel of the Rotor- Gene 2000/3000/6000 (the 1st position must contains reaction tube with reagents). Close the window *Auto Gain Calibration Setup.*

RESULTS ANALYSIS:

1. Press *Analysis* then select button *Quantitation* and press *Show*. For channel Fam (Green) select in the window *Quantitation Analysis Threshold 0,03, Dynamic Tube*, *Slope Correct*, *More Setting (Outlier Removal) and NTC Threshold 10%* . Perform this operation for JOE (Yellow) channel.
2. The results are interpreted with the software of Rotor-Gene 2000/3000/6000 through the presence of crossing of fluorescence curve with the threshold line. β-globine gene (IC) is detected on the FAM (Green) channel, *Pneumocystis jirovecii (carinii)* on the JOE (Yellow) channel.
3. The sample is considered to be positive for *Pneumocystis jirovecii (carinii)* if in the channel Joe (Yellow) the value of **Ct** is different from zero (Ct < 32)
4. The sample is considered to be negative if in the channel Joe (Yellow) the Ct value is not determined (the fluorescence curve does not cross the threshold line) and in the results table on the channel Fam (Green) the Ct value is lower than 28.

Thermal cycle program using iQ iCycler (Biorad):

Cycle	Repeats	Step	Dwell Time	Set Point
1	1	1	15:00	95.0
2	45	1	00:20	95.0
		2	01:00	60.0*

*Fluorescence is measured at 60°C on FAM-490 and HEX-530

Thermal profile for MX3005P (Stratagene):

95°C – 15 min
95°C – 20 sec
60°C – 60 sec*
***Cycle Repeats* – 45 times**
*Fluorescence is measured at 60°C on FAM and HEX channels

Thermal cycle program using SmartCycler (Cepheid)

Stage	Temp	Secs	Optics	Repeat
Stage 1. Hold	95°C	900	Off	1
Stage 2	95°C	15	Off	45
3-Temperature Cycle	60°C	45	ON	

*Fluorescence is measured at 60°C on FAM and JOE/HEX channels

TROUBLESHOOTING

1. Absent signal of the IC (Fam (Green) channel): retesting of the sample is required.

• The PCR was inhibited. Make sure that you use a recommended DNA extraction method and follow the manufacturer's instructions.
• The reagents storage conditions didn't comply with the instructions. Check the storage conditions
• The PCR conditions didn't comply with the instructions. Check the PCR conditions and for the IC detection select the fluorescence channel reported in the protocol.
• No correct sample collection or preparation.

2. No signal on the Joe (Yellow)/Cy3/HEX and Fam (Green) channels with Positive Control.

• The reagents storage conditions didn't comply with the instructions. Check the storage conditions
• The PCR conditions didn't comply with the instructions. Check the temperature profile and select the fluorescence channel reported in the protocol.
• Incorrect configuration of the PCR reaction: Check the reagents preparation step.

3. Any signal with Negative Control.

• Contamination during PCR preparation procedure. All samples results are invalid. Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents. Pipette the Positive controls at the end. Repeat the PCR preparation with the new set of reagents.

EXPLANATION OF SYMBOLS

Catalogue Number

For *in Vitro* Diagnostic Use

Lot Number


Expiration Date

Contains reagents


Caution!

Version


Manufacturer


Temperature limitation

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