Instruction for Use

diarellaP.jirovecii quant

real time PCR Kit

For *in vitro* detection and quantification of the DNA of Pneumocystis jirovecii extracted from biological specimens.

REF **G01139-96**

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- 18°C

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Index

1 Intended Purpose

The Kit is designed for the detection and quantification of the nucleic acid of Pneumocystis jirovecii in eluates from biological specimens. The assay is an in vitro diagnostic medical device and intended to be used by professional users in a laboratory environment. It can be performed manually or using an automated platform. The assay serves as an aid in the diagnosis of Pneumocystis pneumonia (PCP) and resident flora of Pneumocystis jirovecii. It also serves as an aid in follow up and monitoring of PCP patients.

2 Pathogen Information

Pneumocystis jirovecii is a yeast-like fungus and the causative agent of Pneumocystis pneumonia (PCP) in humans. While Pneumocystis jirovecii is able to colonize healthy people and is found widely spread among the normal population, it can cause serious problems in immunocompromised patients, like HIV patients, patients receiving organ transplants or chemotherapy-treated patients. In those patients, it can cause a PCP with symptoms like dyspnoea, fever, cough and in untreated cases, a very high mortality rate.
Different studies suggest an airborne transmission of Pneumocystis jirovecii with a short period of exposition and a low copy number of organisms.

3 Principle of the Test

The diarellaP.jirovecii quant real time PCR kit contains specific primers and dual-labelled probes for the amplification of the DNA of Pneumocystis jirovecii extracted from biological specimens. The PCR targets sequences of the mitochondrial transcribed large subunit rRNA gene (mtLSU) in biological samples [1].
The presence of nucleic acids is detected by an increase in fluorescence due to hydrolysis of the probes during amplification. The fluorescence of the pathogen-specific probe is measured in the FAM channel.
Furthermore, diarellaP.jirovecii quant real time PCR kit contains a Control DNA (Internal Process Control, IPC), which is added during DNA extraction and detected in the same reaction by a HEX-labelled probe.
The Control DNA allows the detection of PCR inhibition and acts as control, that the nucleic acids were isolated from the biological specimen.
Additionally, the diarellaP.jirovecii quant real time PCR Kit contains an Internal System Control (ISC). The ISC consists of primers and probes for the detection of a house keeping gene (Human Succinate Dehydrogenase) in the

eluate from a biological specimen. The ISC helps preventing false negative results due to insufficient sample collection or transport. The amplification of the Human Succinate Dehydrogenase target sequence is measured in the ROX channel.

4 Package Contents

The reagents supplied are sufficient for 96 reactions.

Table 1: Components of the diarellaP.jirovecii quant real time PCR Kit

Label	Lid Colour	Content
Reaction Mix	yellow	1 x 1344 µl
Positive Control	red	1 x 150 µl
Negative Control	green	1 x 150 µl
Control DNA	colourless	1 x 480 µl
Standard 1	black	1 x 150 µl
Standard 2	violett	1 x 150 µl
Standard 3	orange	1 x 150 µl

5 Equipment and Reagents to be Supplied by User

- DNA isolation kit (e.g. NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- Optional: Extraction Instrument for automation
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Tabletop centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

6 Transport, Storage and Stability

The diarellaP.jirovecii quant real time PCR Kit is shipped on dry ice or cool packs. All components must be stored at maximum -18°C in the dark immediately after arrival. Do not use reagents after the date of expiry printed on the package. Up to 20 freezing and thawing cycles are possible. Protect kit components from direct sunlight during the complete analysis.

7 Warnings and Precautions

Read the Instruction for Use carefully before using the product.
Before first use check the product and its components for:

- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipet tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organisations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.

- Discard sample and assay waste according to your local safety regulations.
- Do not combine kit components of different lot numbers.

8 Sample Material

Starting material for diarellaP.jirovecii quant real time PCR is DNA isolated from biological specimens.
By its nature as a lung pathogen, P. jirovecii can be found in samples like human broncho alveolar lavage liquids (BALF). The usage of DNA isolates from tracheal secretions or induced sputum samples is also possible.

9 Sample Preparation

Commercial kits for DNA isolation such as the following are recommended:

- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

Please follow the Instruction for Use of the respective extraction kit.

Important:
In addition to the samples always run a ‚water control' in your extraction. Treat this water control analogous to a sample.
Comparing the amplification of the Control DNA in the samples to the amplification of the Internal Control in the water control will give insights on possible inhibitions of the real time PCR. Furthermore, possible contaminations during DNA extraction will be detectable.

Please note the chapter 'Control DNA'
If the real time PCR is not performed immediately, store extracted DNA according to the instructions given by the manufacturer.

10 Control DNA

A Control DNA is supplied as extraction control. This allows the user to control the DNA isolation procedure and to check for possible real time PCR inhibition.

Add 5 µl Control DNA per extraction (5 µl x (N+1)). Mix well. Perform the DNA isolation according to the manufacturer's instructions.

The Control DNA must be added to the Lysis Buffer of the extraction kit.

11 Real time PCR

11.1 Important Points Before Starting

- Please pay attention to the chapter 7 ‚Warnings and Precautions'.
- Before setting up the real time PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed, and centrifuged very briefly.

11.2 Procedure

The Master Mix contains all of the components needed for PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the Master Mix

Volume per Reaction	Volume Master Mix
14.0 µl Reaction Mix	14.0 µl x (N+1)

Real time PCR set-up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument / take an optical PCR reaction plate.
- Pipet **14 µl** of the Master Mix into each optical PCR reaction tube / the optical PCR reaction plate.
- Add **6 µl** of the eluates from the DNA isolation (including the eluate of the water control), the Positive Control and the Negative Control to the corresponding optical PCR reaction tube / the optical PCR reaction plate (Table 3).
- In case of quantitative analysis, prepare 3 extra reactions and add **6 µl** of Standard 1, Standard 2 and Standard 3 to the corresponding optical PCR reaction tube / the optical PCR reaction plate (Table 3).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 3: Preparation of the real time PCR

Component	Volume
Master Mix	14.0 µl
Sample	6.0 µl
Total volume	20.0 µl

11.3 Instrument Settings

For the real time PCR use the thermal profile shown in Table 4.

Table 4: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	45
Annealing and Extension	40 sec	60°C	
	Acquisition at the end of this step		

If in the same run samples should be tested for pathogens with RNA genome, use the thermal profile shown in Table 5.

Table 5: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Reverse Transcription	10 min	45°C	1
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	45
Annealing and Extension	40 sec	60°C	
	Acquisition at the end of this step		

Dependent on the real time instrument used, further instrument settings have to be adjusted according to Table 6.

Table 6: Overview of the instrument settings required for the diarellaP.jirovecii quant real time PCR.

Real time PCR Instrument	Parameter	Detection Channel	Notes		
LightCycler 480II			Colour Compensation Kit Multiplex 1 (G070MP1-CC) required		
			Melt Factor	Quant Factor	Max Integration Time (sec)
	P. jirovecii	465-510	1	10	1
	Control DNA (IPC)	533-580	1	10	2
	ISC	533-610	1	10	2
Stratagene Mx3000P / Mx3005P	P. jirovecii	FAM	Gain 8	Reference Dye: None	
	Control DNA (IPC)	HEX	Gain 1		
	ISC	ROX	Gain 1		
QuantStudio 5 CFX96 Touch CFX Opus 96 NEOS-48 NEOS-96 AriaMx qTower^{3}G	P. jirovecii	FAM	Option Reference Dye ROX: NO		
	Control DNA (IPC)	HEX			
	ISC	ROX			
Mic qPCR Cycler	P. jirovecii	Green	Gain 8		
	Control DNA (IPC)	Yellow	Gain 10		
	ISC	Red	Gain 10		
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	P. jirovecii	Green	Gain 5	Outlier Removal NTC Threshold 15%	
	Control DNA (IPC)	Yellow	Gain 5		
	ISC	Orange	Gain 5		

Other programable real time PCR Instruments may be used. For assistance, please contact our scientists at info@gerbion.com.

12 Data Analysis

Following results can occur:

Interpretation			
FAM Channel P. jirovecii	**ROX Channel** ISC	**HEX Channel** IPC	
positive	positive or negative[1]	positive or negative[2]	**Positive result, the sample contains P. jirovecii DNA.**
negative	positive	≤ 34 [3]	**Negative result, the sample contains no P. jirovecii DNA.**
negative	negative or > 35	≤ 34 [3]	**No diagnostic statement can be made.** The sample contains no / very low amount of human DNA.
negative	positive or negative[1]	negative or > 34 [3]	**No diagnostic statement can be made.** The real time PCR is either inhibited or errors occurred while DNA extraction.

1 If the analysed samples originate from cultivation, the ROX channel may be negative.

2 A strong positive signal in the FAM channel can inhibit the IPC. In such cases the result for the Control DNA can be neglected.

3 In case of high C_T values, the IPC should be compared to the water control as described in the chapter 'Assay validation'.

Figure 1, Figure 2 and Figure 3 show examples for positive and negative real time PCR results.

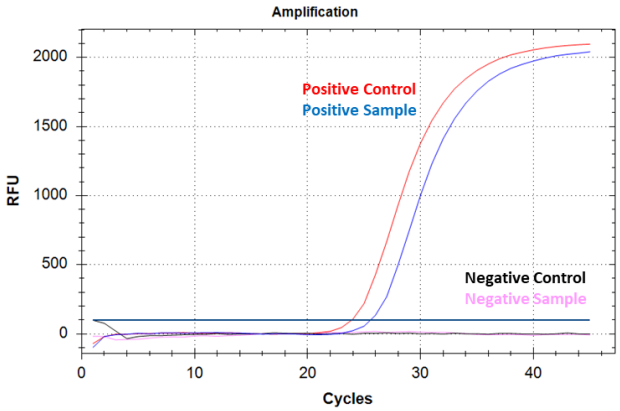

Figure 1: The figure shows the C_T values of eluates from human bronchoalveolar lavage fluid (BALF) samples after nucleic acid extraction using NukEx Mag RNA/DNA nucleic acid extraction Kit. The Positive Sample shows pathogen specific amplification in the FAM channel (Positive Sample and Positive Control), whereas no fluorescence signal is detected in the Negative Sample or the Negative Control (CFX96 Touch).

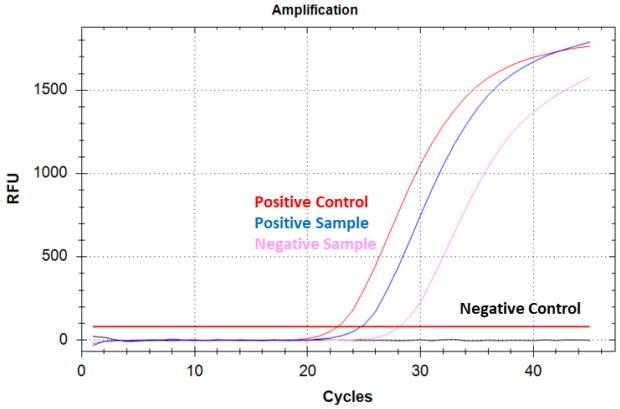

Figure 2: Signals of the amplification of the ISC in the ROX channel. The figure shows the C_T values of eluates from human bronchoalveolar lavage fluid (BALF) samples after nucleic acid extraction using NukEx Mag RNA/DNA nucleic acid extraction Kit (CFX96 Touch).

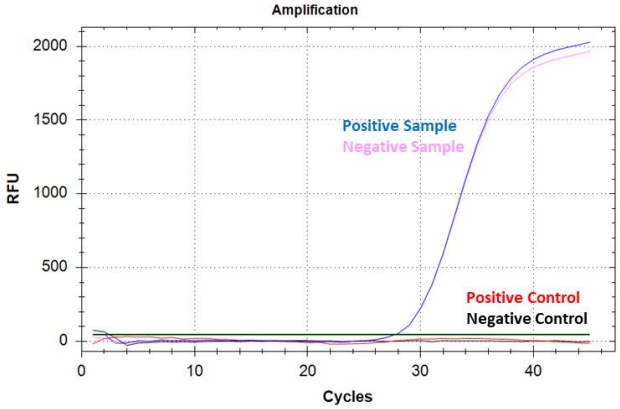

Figure 3: The Positive Sample and the Negative Sample show a signal in the Control DNA specific HEX channel (IPC). The amplification signal of the Control DNA in the Negative Sample shows that the missing signals in the pathogen specific channel FAM is not due to PCR inhibition or failure of DNA isolation, but that the sample is a true Negative Sample (CFX96 Touch).

12.1 Quantitative Analysis

The diarellaP.jirovecii quant real time PCR Kit includes three Quantification Standards (Table 7). The calculation of the fungal load can be done manually or, in most PCR cyclers, directly in the instrument software by the definition of quantification standards (Figure 5).

Table 7: Quantification standards for the P. jirovecii LSU gene.

Quantification Standard	Concentration of the Standard [copies/µl]
Standard 1	1000
Standard 2	100
Standard 3	10

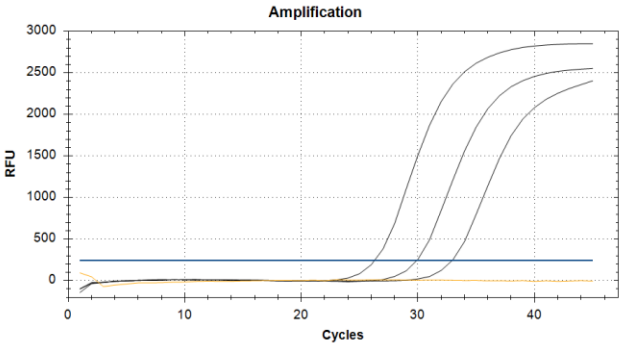

Figure 4: The amplification curves of the three standards for Pneumocystis jirovecii LSU DNA (FAM channel) with 1000 copies per µl (Standard 1), 100 copies per µl (Standard 2) and 10 copies per µl (Standard 3).

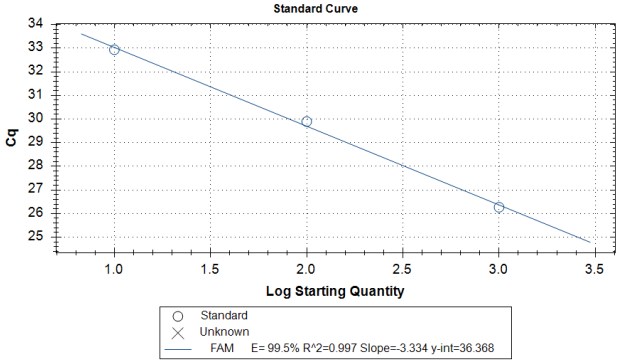

Figure 5: The C_T values of three standards for Pneumocystis jirovecii LSU DNA (FAM channel) plotted against the target copies per µl.

$$C_T = a * \log (N) + b$$

$$N = 10^{\frac{(C_T - b)}{a}}$$

C_T	Threshold Cycle
a	Slope
N	copy number [copies/µl]
b	Intercept

13 Assay Validation

Negative Control
The Negative Control must show no C_T in the FAM, ROX and HEX channel.

Positive Control
The Positive Control must show a positive (i.e. exponential) amplification curve in the different channels FAM and ROX. The Positive Control must fall below a C_T of 30.

Internal Controls
The following values for the amplification of the Internal Controls are valid using gerbion nucleic acid extraction kit NukEx Mag RNA/DNA. All Internal Controls (ISC and IPC, seqc – sample and extraction quality control) must show a positive (i.e. exponential) amplification curve.
The Control DNA (IPC) must fall below a C_T of 34. If the Control DNA is above C_T 34 this points to a DNA purification problem or a strong positive sample (FAM channel positive) that can inhibit the IPC. In the latter case, the assay is valid. It is recommended to perform the extraction of a water control in each run. The IPC in the water control must fall below a C_T of 34.
For most bronchoalveolar lavage fluid (BALF) samples, the ISC shows C_T values from app. 15 to app. 30. A heavily delayed signal of higher than a C_T of 34 indicates a low sample amount or low sample quality. Therefore, false-negative results cannot be ruled out. In case of no amplifications in the FAM channel, there must be an amplification curve in the ROX channel (ISC) and the HEX (IPC) channel when using eluates of primary samples from humans.
If other nucleic acid extraction kits are used, the customer must define own cut offs. In this case the C_T value of the Control DNA (IPC) in an eluate from a sample should not be delayed for more than 4 C_T in comparison to an eluate from an extracted water control.

Pathogen Quantification
The kit includes three quantitative standards with known copy numbers of pathogen target sequences. If a positive signal for a sample is detected above the C_T value of Standard 3 presumably detection of the resident flora is observed.

14 Limitations of the Method

- Strict compliance with the Instruction for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false-negative or invalid results.
- As with any diagnostic test, results of the diarellaP.jirovecii quant real time PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

15 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

No fluorescence signal in the FAM or ROX channel of the Positive Control	
The selected channel for analysis does not comply with the protocol	Select the FAM channel for analysis of the Pneumocystis jirovecii specific amplification, the ROX channel for the amplification of the ISC and the HEX channel for the amplification of the Control DNA (IPC).
Incorrect configuration of the real time PCR	Check your work steps and compare with chapter 'Procedure'.
The programming of the thermal profile is incorrect	Compare the thermal profile with the protocol in chapter 'Instrument Settings'.
Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability'.
Weak or no signal of the Control DNA (IPC) and ISC and simultaneous absence of a signal in the pathogen specific FAM channel.	
real time PCR conditions do not comply with the protocol	Check the real time PCR conditions (chapter 'Real time PCR').
real time PCR inhibited	Make sure that you use an appropriate isolation method (see chapter 'Sample Preparation') and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed.
sample material not sufficient	Make sure that enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter 'Sample Preparation') and follow the manufacturer's instructions.
DNA loss during isolation process	In case the Control DNA was added before extraction, the lack of an amplification signal can indicate that the DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability'.

Detection of a fluorescence signal in the FAM channel of the Negative Control	
Contamination during preparation of the PCR	Repeat the real time PCR in replicates. If the result is negative in the repetition, the contamination occurred when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time PCR.
Detection of a fluorescence signal in the ROX channel of the Negative Control	
Contamination with human DNA during preparation of the real time PCR	As long as the ROX channel shows very high C_T values (C_T > 35), the contamination is negligible. If the FAM channel is negative in the Negative Control, the PCR is still valid for the detection of P. jirovecii.

16 Kit Performance

16.1 Analytical Sensitivity

For the FAM channel the limit of detection (LoD) of diarellaP.jirovecii quant real time PCR Kit was determined using serial dilutions of the synthetic DNA fragments of the Pneumocystis jirovecii LSU gene. For the ROX channel synthetic DNA fragments of the Human Succinate Dehydrogenase were used. The determination of the LoD was done on a CFX96 Touch cycler (Bio-Rad).

The LoD of diarellaP.jirovecii quant real time PCR Kit is $\leq$ 2.5 genome copies per µl for the FAM and the ROX channel.

16.2 Analytical Specificity

The specificity of the diarellaP.jirovecii quant real time PCR was evaluated with different other relevant pathogens found in biological samples and basing on in silico analyses.

The results for the sample analysis are shown in Table 8. The results for the in silico analysis are shown in Table 9.

Table 8: Eluted DNA/RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of diarellaP.jirovecii quant real time PCR Kit (FAM channel).

Eluates with known status	**expected result FAM channel**	**diarellaP.jirovecii quant FAM channel**
Adenovirus	negative	negative
Bacillus anthracis	negative	negative
Bordetella bronchiseptica	negative	negative
Bordetella holmesii	negative	negative
Bordetella parapertussis	negative	negative
Bordetella pertussis	negative	negative
Chlamydia pneumoniae	negative	negative
Coxiella burnetii	negative	negative
Cytomegalievirus (CMV)	negative	negative
Epstein Barr Virus (EBV)	negative	negative
Haemophilus influenzae	negative	negative
Helicobacter pylori	negative	negative
Herpes simplex Virus 1 (HSV 1)	negative	negative
Herpes simplex Virus 2 (HSV 2)	negative	negative
Legionella pneumophila	negative	negative
MRSA	negative	negative
Mycobacterium tuberculosis complex	negative	negative
Mycoplasma pneumoniae	negative	negative
Pneumocystis jirovecii	**positive**	**positive**
Pneumocystis sp. 'macacae'	negative	negative
Pseudomonas aeruginosa	negative	negative
Staphylococcus aureus	negative	negative
Streptococcus agalactiae	negative	negative
Varizella Zoster Virus (VZV)	negative	negative

Table 9: Inclusivity of the diarellaP.jirovecii quant real time PCR Kit Primers and Probes (in silico analysis).

241 - 265 sequences (NCBI)		Homology	Comment
P. jirovecii	Forward Primer	241 sequences: 100%	no mismatch
	Reverse Primer	264 sequences: 100%	no mismatch
	Probe	265 sequences: 100%	no mismatch

16.3 Linear Range

The linear range of the diarellaP.jirovecii quant real time PCR Kit was evaluated by analysing logarithmic dilution series of synthetic DNA fragments of the target sequences.

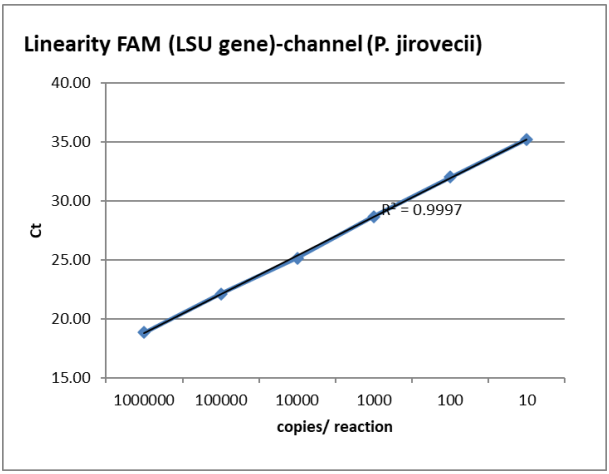

Figure 6: Determination of the linear range of the diarellaP.jirovecii quant real time PCR Kit for the detection of the LSU DNA of Pneumocystis jirovecii in the FAM channel.

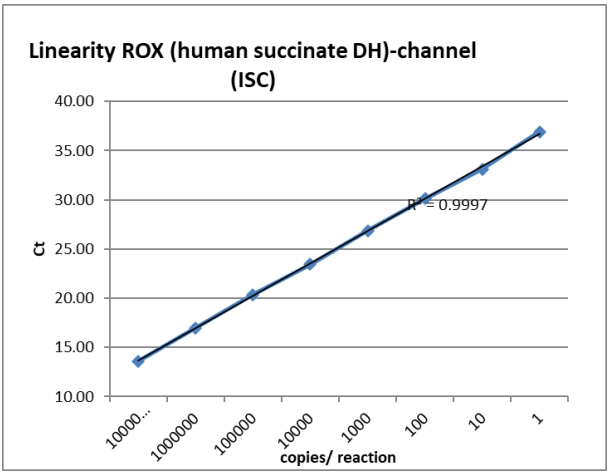

Figure 7: Determination of the linear range of the diarellaP.jirovecii quant real time PCR Kit for the ISC in the ROX channel.

16.4 Precision

The precision of the diarellaP.jirovecii quant real time PCR Kit was determined as intra-assay variability, inter-assay variability and inter-lot variability.

Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of Pneumocystis jirovecii synthetic DNA, ISC specific synthetic DNA and on the threshold cycle of the Control DNA (IPC). The results are shown in Table 10.

Table 10: Precision of the diarellaP.jirovecii quant real time PCR Kit

P. jirovecii (FAM)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.26	0.82
Inter-Assay-Variability	25	0.55	1.72
Inter-Lot-Variability	25	0.28	0.88
ISC (ROX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.23	0.76
Inter-Assay-Variability	25	0.09	0.31
Inter-Lot-Variability	25	0.23	0.77
IPC (HEX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	250	0.18	0.61
Inter-Assay-Variability	250	0.36	1.24
Inter-Lot-Variability	250	0.07	0.23

16.5 Diagnostic Sensitivity

The diagnostic sensitivity of real time (RT-) PCR assays is mainly dependent on the DNA/RNA extraction method used to isolate DNA and RNA from various biological specimens. DNA/RNA extraction reagents are not part of the gerbion real time (RT-) PCR kits. gerbion real time (RT-) PCR kits include an extraction control and guidelines for the validation criteria of the extraction control in each reaction. The extraction control indicates inhibition of the real time (RT-) PCR and/or inefficient nucleic acid extraction. It cannot be used as a calibrator.

Therefore, gerbion guarantees the analytical sensitivities and specificities of the real time (RT-) PCR kits, performed with eluted DNA and RNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. gerbion does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of gerbion real time (RT-) PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods. It is the responsibility of the user to qualify the extraction methods used for DNA/RNA isolation from biological samples.

17 Abbreviations and Symbols

LSU	Large subunit of ribosomal DNA	REF	Catalog number
PCP	Pneumocystis pneumonia	Σ	Content sufficient for <n> tests
DNA	Deoxyribonucleic Acid	- 18°C	Upper limit of temperature
PCR	Polymerase Chain Reaction		Manufacturer
REACTION MIX	Reaction Mix		Use by YYYY-MM-DD
CONTROL +	Positive Control	LOT	Batch code
CONTROL −	Negative Control	CONT	Content
CONTROL DNA IPC	Control DNA (IPC)		Consult Instruction for Use
STANDARD 1	Standard 1	IVD	*In vitro* diagnostic medical device
STANDARD 2	Standard 2	CE	European Conformity
STANDARD 3	Standard 3	UDI	Unique Device Identification

18 Literature

[1] Valero et al., (2016) Copy Number Variation of Mitochondrial DNA Genes in Pneumocystis jirovecii According to the Fungal Load in BAL Specimen. frontiers in Microbiology. Sept. 16, Vol. 7, Article 1413