# RIDA®GENE Pneumocystis jirovecii

REF PG1905

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## 1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Pneumocystis jirovecii is a multiplex real-time PCR for the direct qualitative and quantitative detection of *Pneumocystis jirovecii* from human bronchoalveolar lavage fluid (BAL).[1,2]

The RIDA®GENE Pneumocystis jirovecii multiplex real-time PCR is intended to use as an aid in diagnosis for respiratory infections caused by *Pneumocystis jirovecii*.

## 2. Summary and explanation of the test

*Pneumocystis jirovecii* (former *P. carinii*) belongs to the family of *Pneumocystidaceae* and may lead to an interstitial pneumonia. Opportunistic infections are a major problem in immunocompromised patients, for example HIV/AIDS patients, chemotherapy-treated patients and patients receiving an organ transplant. *Pneumocystis jirovecii* causes respiratory infections and is the most common opportunistic illness in HIV-infected people. *Pneumocystis jirovecii* does not cause any harm in healthy people and is widely spread among the normal population. However, immunocompromised people infected with *Pneumocystis jirovecii* develop pneumonia with symptoms including dry cough, shortness of breath, tachypnea and fever.[3] Although HAART therapy decreased the *Pneumocystis jirovecii* incidence by 3.4 % per year after 1996, it is estimated that still 9 % among hospitalized HIV/AIDS patients and 1 % among solid organ transplant recipients are infected.[4] According to the Center for Disease Control (CDC), *Pneumocystis jirovecii* causes 100 % mortality in patients without treatment and the mortality rate in immunocompromised patients is between 5 % - 40 % in treated patients.[4] The mortality from *Pneumocystis jirovecii* in HIV-uninfected patients can be as high as 40 %.[5] Until now, detection of *Pneumocystis jirovecii* was done by immunofluorescence staining. However, due to its low sensitivity this is now substituted by PCR.[2]

## 3. Test principle

RIDA®GENE Pneumocystis jirovecii is a multiplex real-time PCR for the direct, qualitative and quantitative detection of *Pneumocystis jirovecii* from human bronchoalveolar lavage fluid (BAL).
After DNA isolation, amplification of the gene fragment (if present) specific for *Pneumocystis jirovecii* (mt LSU; large subunit) occurs. The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step, the Taq-polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal, which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. With the standards, Standard A, Standards B and Standards C, included in the kit, it

is possible to quantify the results. The RIDA®GENE Pneumocystis jirovecii multiplex real-time PCR kit contains an Internal Control DNA (ICD) that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.

## 4. Reagents provided

**Tab. 1:** Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

| Kit Code | Reagent | Amount | | Lid Color |
|---|---|---|---|---|
| **1** | Reaction Mix | 2x | 1050 µl | yellow |
| **2** | Taq-Polymerase | 1x | 80 µl | red |
| **D** | Internal Control DNA | 2x | 1700 µl | orange |
| **N** | No Template Control | 1x | 450 µl | white |
| **P** | Positive Control | 1x | 200 µl | blue |
| **10^1** | Standard A | 1x | 100 µl | dark blue |
| **10^3** | Standard B | 1x | 100 µl | dark blue |
| **10^5** | Standard C | 1x | 100 µl | dark blue |

## 5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

## 6. Additional necessary reagents and necessary equipment

The RIDA®GENE Pneumocystis multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

**Tab. 2**: Necessary equipment

| **Extraction platforms** | |
|---|---|
| R-Biopharm | RIDA®Xtract |
| Promega | Maxwell® RSC |
| **Real-time PCR instruments** | |
| Roche | LightCycler® 2.0, LightCycler® 480II |
| Agilent Technologies | Mx3005P |
| Applied Biosystems | ABI 7500 |
| Bio-Rad | CFX96™ |
| QIAGEN | Rotor-Gene Q |

**Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).**

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit II (PG0002) for use with the LightCycler® 2.0
- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 - 20 µl, 20 - 200 µl, 100 - 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (nuclease-free)

## 7. Precautions for users

For *in-vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.

- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

## 8. Collection and storage of samples

### 8.1 Sample preparation from bronchoalveolar lavage (BAL)

For DNA isolation from bronchoalveolar lavage (BAL), use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.

The RIDA®GENE Pneumocystis jirovecii assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

## 9. Test procedure

### 9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each assay run.

We recommend to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control, the Internal Control DNA and Standard A, Standard B and Standard C before using. Keep reagents appropriately cold during working step (2 - 8 °C).

**Tab. 3**: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

| Kit code | Master-Mix components | Volume per reaction | 10 reactions (10 % extra) |
|---|---|---|---|
| **1** | Reaction Mix | 19.3 µl | 212.3 µl |
| **2** | Taq-Polymerase | 0.7 µl | 7.7 µl |
| | **Total** | **20 µl** | **220 µl** |

Mix the components of the Master-Mix gently and briefly spin down.

**Tab. 4**: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

| Kit code | Master-Mix components | Volume per reaction | 10 reactions (10 % extra) |
|---|---|---|---|
| **1** | Reaction Mix | 19.3 µl | 212.3 µl |
| **2** | Taq-Polymerase | 0.7 µl | 7.7 µl |
| **D** | Internal Control DNA | 1.0 µl | 11 µl |
| | **Total** | **21.0 µl** | **231.0 µl** |

Mix the components of the Master-Mix gently and briefly spin down.

### 9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

**Negative control:** Add 5 µl No Template Control to the pre-pipetted Master-Mix.

**Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the negative control.**

**Sample:** Add 5 µl DNA extract to the pre-pipetted Master-Mix.

**Positive control:** Add 5 µl Positive Control to the pre-pipetted Master-Mix.

**Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the positive control.**

**Standard (A, B, C):** Add 5 µl Standard (A, B, C) to the pre-pipetted Master-Mix.

**Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the standards.**

**Note: Using the following cyclers requires to include a standard curve in each run: ABI 7500 (Applied Biosystems) and CFX96™ (Bio-Rad).**

**For all other cyclers, only one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run. Here, the application of a standard curve is only required to run once per lot number.**

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument set-up (see Tab. 5, Tab. 6, Tab. 7, Tab. 8).

## 9.3 PCR instrument set-up

### 9.3.1 DNA real-time PCR profile

**Tab. 5**: DNA real-time PCR profile for LightCycler® series and Rotor-Gene Q

| | |
|---|---|
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 10 sec, 95 °C<br>15 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and Extension occur in the same step.**

**Tab. 6**: DNA real-time PCR profile for Mx3005P, ABI7500, CFX96™

| | |
|---|---|
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 15 sec, 95 °C<br>30 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and Extension occur in the same step.**

**Note: The total copy number per reaction of Standard A, Standard B and Standard C has to be typed in into the setup file of the software program of the respective real-time PCR cycler. A total volume of 5 µl DNA is used resulting in following concentrations:**

**Standard A: 5 x $10^1$ copies/reaction**

**Standard B: 5 x $10^3$ copies/reaction**

**Standard C: 5 x $10^5$ copies/reaction**

**Note: The standard curve can be saved on the real-time PCR cycler for each parameter. Apart from the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler, the standard curve is only required to run once per lot number. Using the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler requires to include a standard curve in each run. For all other cyclers, only one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run. The general recommendation is, however, to test always all three standards with every run.**

### 9.3.2 Universal real-time PCR profile

**Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RNA real-time PCR assays in one run.**

**Tab. 7**: Universal real-time PCR profile for LightCycler® series

| | |
|---|---|
| Reverse Transcription | 10 min, 58 °C |
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 10 sec, 95 °C<br>15 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and Extension occur in the same step.**

**Tab. 8**: Universal real-time PCR profile for Mx3005P, ABI7500, CFX96™ and Rotor-Gene Q

| | |
|---|---|
| Reverse Transcription | 10 min, 58 °C |
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 15 sec, 95 °C<br>30 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and Extension occur in the same step.**

**Note: The total copy number per reaction of Standard A, Standard B and Standard C has to be typed in into the setup file of the software program of the respective real-time PCR cycler. A total volume of 5 µl DNA is used resulting in following concentrations:**

**Standard A: 5 x $10^1$ copies/reaction**

**Standard B: 5 x $10^3$ copies/reaction**

**Standard C: 5 x $10^5$ copies/reaction**

**Note: The standard curve can be saved on the real-time PCR cycler for each parameter. Apart from the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler, the standard curve is only required to run once per lot number. Using the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler requires to include a standard curve in each**

**run. For all other cyclers, only one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run. The general recommendation is, however, to test always all three standards with every run.**

## 9.4 Detection channel set-up

**Tab. 9**: Selection of appropriate detection channels

| Real-time PCR Instrument | Detection | Detection Channel | Note |
|---|---|---|---|
| **Roche LightCycler® 2.0** | *Pneumocystis jirovecii* | 530 | **RIDA®GENE Color Compensation Kit II (PG0002) is required** |
| | ICD | 560 | |
| **Roche LightCycler® 480II** | *Pneumocystis jirovecii* | 465/510 | **RIDA®GENE Color Compensation Kit IV (PG0004) is required** |
| | ICD | 533/580 | |
| **Agilent Techn. Mx3005P** | *Pneumocystis jirovecii* | FAM | **Check that reference dye is none** |
| | ICD | HEX | |
| **ABI 7500** | *Pneumocystis jirovecii* | FAM | **Check that passive reference option ROX is none** |
| | ICD | VIC | |
| **Bio-Rad CFX96™** | *Pneumocystis jirovecii* | FAM | **-** |
| | ICD | VIC | |
| **Qiagen Rotor-Gene Q** | *Pneumocystis jirovecii* | Green | **The gain settings have to be set to 5, according to the default settings** |
| | ICD | Yellow | |

## 10. Quality control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer's instructions. Positive control and negative control have to show correct results (see Table 10, Fig. 1) in order to determine a valid run.

The Positive Control has a concentration of $10^3$ copies/µl. In each PCR run it is used in a total amount of 5 x $10^3$ copies.

**Tab. 10**: For a valid run, the following conditions must be met:

| Sample | Assay result | ICD Ct | Target Ct |
|---|---|---|---|
| Positive control | Positive | NA *[1] | See Quality Assurance Certificate |
| Negative control | Negative | Ct > 20 | 0 |

*[1] *No Ct value is required for the ICD to make a positive call for the positive control.*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid, prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

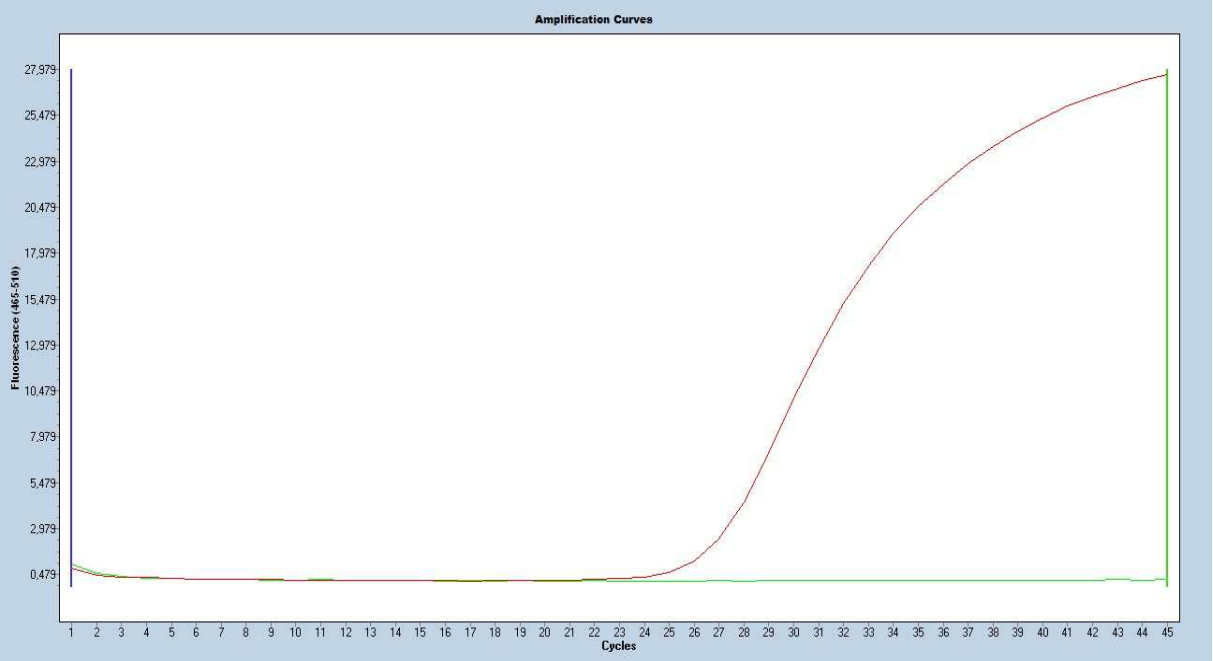


**Fig. 1:** Correct run of the positive control (red) and negative control (green) (*Pneumocystis jirovecii*) on the LightCycler® 480II

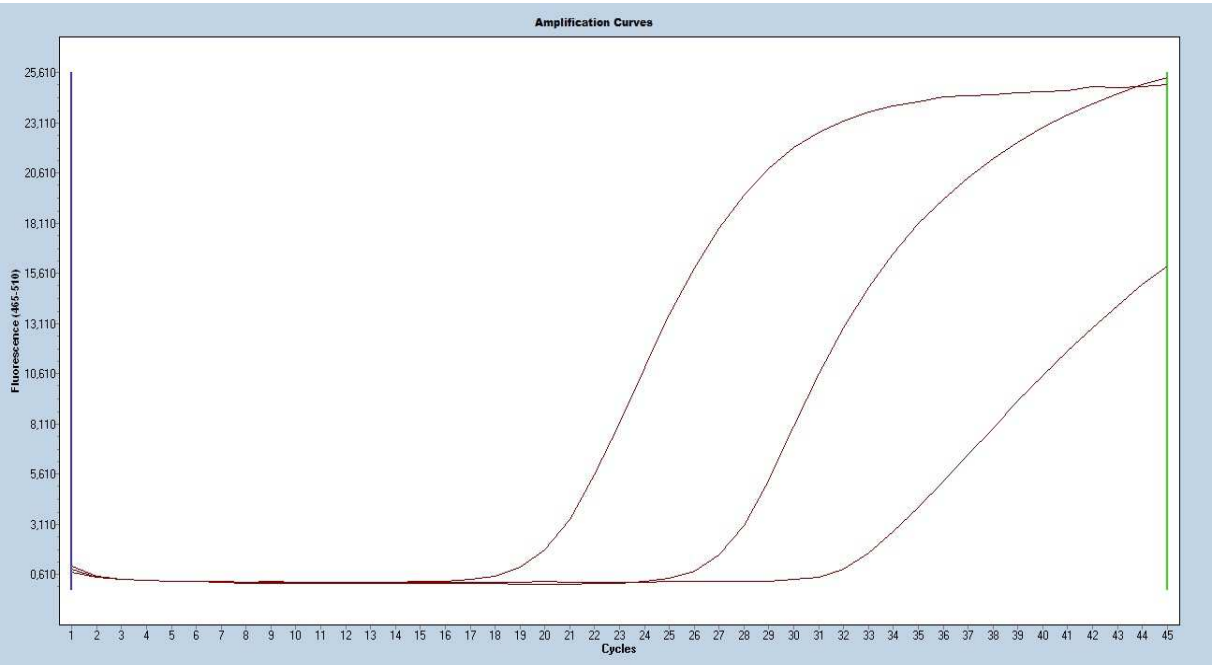


**Fig. 2**: Standard series *Pneumocystis jirovecii* with Standard A ($10^1$ DNA copies per µl), Standard B ($10^3$ DNA copies per µl) and Standard C ($10^5$ DNA copies per µl) on the LightCycler® 480II

### 10.1 Validity of quantitative detection

For the validity of a quantitative diagnostic test run, all control conditions of a valid qualitative diagnostic test run must be met. Furthermore, for accurate quantification results a valid standard curve has to be generated. For a valid quantitative diagnostic test run, the following control parameter values of the standard curve should be achieved.

| | Control parameter | Valid value |
|---|---|---|
| **Roche LightCycler® 2.0** | Efficiency | 1,9 – 2,1 |
| **Roche LightCycler® 480II** | Efficiency | 1,9 – 2,1 |
| | Slope | -3,1 – -3,6 |
| **Agilent Techn. Mx3005P** | Rsq | > 0,98 |
| | Slope | -3,1 – -3,6 |
| **ABI 7500** | R^2 | > 0,98 |
| | Slope | -3,1 – -3,6 |
| **Bio-Rad CFX96™** | R^2 | > 0,98 |
| | Slope | -3,1 – -3,6 |
| **Qiagen Rotor-Gene Q** | R^2 | > 0,98 |
| | M | -3,1 – -3,6 |

## 11. Result interpretation

The result interpretation is done according to Table 11.

**Tab. 11**: Sample interpretation

| Target genes | | |
|---|---|---|
| ***Pneumocystis jirovecii*** | **ICD** | **Ergebnis** |
| **positive** | **positive/negative** | ***Pneumocystis jirovecii* detected** |
| negative | **positive** | **Target genes not detected** |
| negative | negative | **Invalid** |

*Pneumocystis jirovecii* is detected, if the sample DNA and the Internal Control DNA show an amplification signal in the detection system.

*Pneumocystis jirovecii* is also detected, if the sample DNA shows an amplification signal but none for the Internal Control DNA in the detection system. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA

*Pneumocystis jirovecii* is not detected, if the sample DNA shows no amplification signal, but an amplification signal for the Internal Control DNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA

A sample is invalid, if the sample DNA and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

### 11.1 Quantification of samples

To quantify *Pneumocystis jirovecii* positive samples, a standard curve with the Standard A, Standard B and Standard C has to be performed separately. The standard curve measurement has to be saved separately. However, the same standard curve measurement can be used in all runs with products from the same lot number by importing the saved experiment.

**Note: This is not valid for the following cyclers: ABI 7500 (Applied Biosystems) and CFX96™ (Bio-Rad). Here, a standard curve hast to be measured with each run.**

**For all other cyclers, one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run.**

To quantify *Pneumocystis jirovecii* positive samples, all standard samples (A, B and C), the positive control and the negative control as well as the unknown samples to

be quantified, have to be selected and analyzed according to the instructions of the cycler manufacturer. Correct quantification results are only reliable if Ct-values of the *Pneumocystis jirovecii* specific target gene (mt LSU; large subunit) can be detected within the standard Ct-range.

With the quantitative RIDA®GENE Pneumocystis jirovecii multiplex real-time PCR the amount of DNA in copies/reaction of the parameter is calculated. The conversion in copies/ml is done with a correction factor K and takes into account the dilutions of the extraction procedure (dependent on the extraction kit used) and the PCR set-up.

The conversion of the result of the quantitative RIDA®GENE Pneumocystis jirovecii multiplex real-time PCR in copies/ml is calculated with following formula:

**C [copies/ml] = c [copies/reaction] x K**

C [copies/ml] - concentration of sample in copies/ml sample
c [copies/reaction] - DNA concentration in PCR reaction (result of quantitative PCR)
K - correction factor

For the calculation of the correction factor, following information has to be considered:
- Sample dilution
- Starting volume of sample for DNA extraction
- DNA extract from total eluate used for PCR reaction

**Tab. 12**: Example of calculation of correction factor K using the Maxwell® RSC (Promega)

| Description | Factor |
|---|---|
| 300 µl sample put into extraction*, eluted in 60 µl final volume | No factor |
| 5 µl DNA extract put into PCR (total eluate 60 µl =1/12) | X 12 |
| 300 µl sample scaled up to 1 ml* | X 3. $\overline{3}$ |
| Correction factor K for *Pneumocystis jirovecii* | 40 |

** Result is based on 1 ml BAL starting material*

**Note: For further information on quantification of the samples please contact pcr@r-biopharm.de.**

## 12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for human bronchoalveolar lavage fluid (BAL).
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Pneumocystis jirovecii assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of target genes ((mt LSU; large subunit).

## 13. Performance characteristics

### 13.1 Clinical performance

In a retrospective clinical validation study we analyzed 203 extracted specimens (BAL) with the RIDA®GENE Pneumocystis jirovecii assay and an in-house real-time PCR assay in a laboratory in Germany.

**Tab. 13**: Correlation of the *Pneuomocystis jirovecii* results with the RIDA®GENE Pneumocystis jirovecii multiplex real-time PCR and reference in-house real-time PCR.

| | | In-house real-time PCR | | | |
|---|---|---|---|---|---|
| | | Positive | Negative | Total | Comments |
| **RIDA®GENE Pneumocystis jirovecii** | Positive | 28 | 2 [b)] | 30 | Pos. agreement: 91.8 % |
| | Negative | 3 [a)] | 170 | 173 | Neg. agreement: 98.6 % |
| | Total | 31 | 172 | 203 | |

*a) Three (3) samples are below the limit of detection (LOD) of the RIDA®GENE Pneumocystis jirovecii assay with a Cp value > 35 in the reference in-house real-time PCR assay.*

*b) Two (2) samples are below the limit of detection (LOD) of the reference in-house real-time PCR assay with a Cp value > 33 in the RIDA®GENE Pneumocystis jirovecii assay*

## 13.2 Analytical sensitivity

The RIDA®GENE Pneumocystis jirovecii multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction for *Pneumocystis jirovecii*.

The following figure 2 shows a dilution series of *Pneumocystis jirovecii* ($10^5$ - $10^1$ DNA copies per µl) on the LightCycler® 480II.

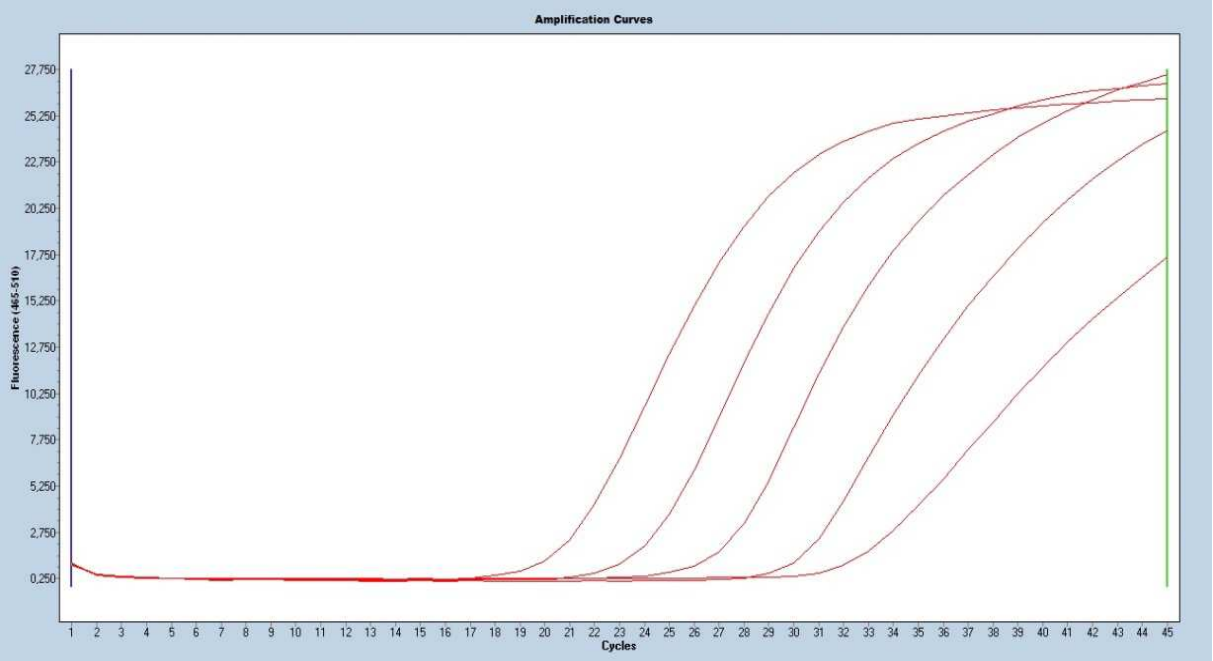


**Fig. 2**: Dilution series *Pneumocystis jirovecii* ($10^5$ – $10^1$ DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

## 13.3 Analytical specificity

The RIDA®GENE Pneumocystis jirovecii multiplex real-time PCR is specific for *Pneumocystis jirovecii*. No cross-reaction could be detected for the following species (see Tab. 14, * detected with sequence alignment):

**Tab. 14**: Cross-reactivity testing

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Acinetobacter baumannii* | - | *Corynebacterium diphtheriae* | - | *Klebsiella pneumoniae* | - | *Rhizomucor pusillus* | - |
| *Alternaria alternata* | - | Coxsackie B4, human | - | *Lactobacillus plantarum* | - | *Saccharomyces cerevisiae* | - |
| Adenovirus 1, human, strain Adenoid 71 | - | Cytomegalovirus, human | - | *Legionella pneumophila* subsp. *pneumophila* | - | *Scedosporium apiospermum* | - |
| Adenovirus 7, human, strain Gomen | - | *Doratomyces microsporus* | - | *Moraxella catarrhalis* | - | *Scedosporium prolificans* | - |
| *Aspergillus fumigatus** | - | Epstein-Barr-Virus, strain B95-8 | - | *Mycoplasma pneumoniae* | - | *Sporothrix schenckii* | - |
| *Aspergillus terreus* | - | *Fusarium solani* | - | *Neisseria meningitidis* | - | *Staphylococcus aureus* | - |
| *Bordetella parapertussis* | - | *Haemophilus influenzae* Rd | - | Parainfluenza virus 1, human strain C35 | - | *Staphylococcus epidermidis* | - |
| *Bordetella pertussis* | - | *Helicobacter felis* | - | Parainfluenza virus 2, human, strain Greer | - | *Staphylococcus haemolyticus* | - |
| *Candida glabrata* | - | Herpes simplex virus 1, strain McIntyre | - | Parainfluenza virus serotype 3 | - | *Staphylococcus hominis* subsp. *novobiosepticus R22* | - |
| *Candida parapsilosis* | - | Herpes simplex virus 2, strain MS | - | Parainfluenza virus 4b, human, strain CH19503 | - | *Streptococcus pneumoniae* | - |
| *Candida tropicalis* | - | *Human Metapneumovirus* | - | Respiratory syncitial virus, human, strain 9320 | - | *Streptococcus pyogenes* | - |
| *Cladosporium* spp | - | Influenza virus, infectious A/PR/8/34 | - | Respiratory syncitial virus, human, strain Long | - | *Streptococcus salivarius* | - |
| Coronavirus 229E, human | - | *Klebsiella oxytoca* | - | Rhinovirus, genogroup A, human | - | Varicella Zoster Virus (Type B) | - |

## 14. Version history

| Version number | Chapter and designation |
|---|---|
| 2019-07-22 | 4. Reagents provided<br>6. Additional necessary reagents and necessary equipment<br>9.2 Preparation of the PCR-Mix<br>9.3 PCR instrument set-up<br>10. Quality control |

## 15. Explanation of symbols

General symbols

| | |
|---|---|
| IVD | For in vitro diagnostic use |
| | Consult instructions for use |
| LOT | Lot number |
| | Expiry |
| | Store at |
| REF | Article number |
| Σ | Number of tests |
| | Date of manufacture |
| | Manufacturer |

Testspecific symbols

Not applicable

## 16. Literature


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