Instructions for Use

RealStar®
Pneumocystis jirovecii PCR Kit 1.0

09/2016  EN
RealStar®

Pneumocystis jirovecii
PCR Kit 1.0

For use with

Mx 3005P™ QPCR System (Stratagene)
VERSANT® kPCR Molecular System AD (Siemens Healthcare)
ABI Prism® 7500 SDS (Applied Biosystems)
ABI Prism® 7500 Fast SDS (Applied Biosystems)
Rotor-Gene® 6000 (Corbett Research)
Rotor-Gene® Q5/6 plex Platform (QIAGEN)
CFX96™ Real-Time System (Bio-Rad)
CFX96™ Deep Well Real-Time System (Bio-Rad)
LightCycler® 480 Instrument II (Roche)
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1. Intended Use

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 is an in vitro diagnostic test, based on real-time PCR technology, for the detection and quantification of Pneumocystis jirovecii specific DNA.

2. Kit Components

<table>
<thead>
<tr>
<th>Lid Color</th>
<th>Component</th>
<th>Number of Vials</th>
<th>Volume [µl/Vial]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>Master A</td>
<td>8</td>
<td>60</td>
</tr>
<tr>
<td>Purple</td>
<td>Master B</td>
<td>8</td>
<td>180</td>
</tr>
<tr>
<td>Green</td>
<td>Internal Control</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>Red</td>
<td>QS1-4*</td>
<td>4</td>
<td>250</td>
</tr>
<tr>
<td>White</td>
<td>Water (PCR grade)</td>
<td>1</td>
<td>500</td>
</tr>
</tbody>
</table>

* The RealStar® Pneumocystis jirovecii PCR Kit 1.0 contains four different Quantification Standards.

3. Storage

- The RealStar® Pneumocystis jirovecii PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer’s instructions and recommendations.

NOTE

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).
5. Background Information

Pneumocystis jirovecii is the causative agent of Pneumocystis pneumonia (PCP), one of the most frequent and severe opportunistic infections in immunocompromised individuals. The symptoms of PCP include dyspnea, nonproductive cough, and fever. In untreated PCP, increasing pulmonary involvement leads to death. Pneumocystis is an obligate extracellular pathogen that exists in trophic and cystic forms. Initially classified as a protozoan, it was reclassified as a fungus based on greater DNA sequence homology with fungal organisms. Pneumocystis organisms represent a large group of species with worldwide distribution and a strong specificity for a given mammalian host species. Historically, all forms of Pneumocystis were referred to as Pneumocystis carinii. In recognition of the specificity of different organisms, the human form was renamed P. jirovecii. P. carinii is now reserved for the rat form of Pneumocystis.

Evidence from different studies suggests that Pneumocystis is transmitted by an airborne route, and transmission requires a short period of exposure and low numbers of organisms. Most human beings become seropositive to P. jirovecii organisms or antigens by the ages of 2 to 4 years. P. jirovecii can be detected in populations without underlying immunosuppression. Colonization, carriage, asymptomatic infection, and subclinical infection have all been used to describe the presence of Pneumocystis organisms or DNA in the absence of PCP. The effects of colonization on the host and in mild respiratory infections, chronic lung disease, and progression to PCP have yet to be determined. Also, it is still under debate, if reactivation of latent infection is the primary cause of PCP or if person to person transmission is a key component of the disease process. According to the reactivation theory, Pneumocystis is commonly encountered in the environment during childhood. The organism does not cause clinical disease but it harbors within the host and can subsequently reactivate to cause PCP, if the host’s immune function declines. On the other hand, it has recently been recognized that de novo exposure either from the environment or from individuals with PCP or colonized with Pneumocystis may result in transmission.

Because Pneumocystis cannot be cultured, diagnosis relies traditionally in visualization of the organism in respiratory samples, including induced sputum, BAL fluid, or lung tissue, or in the specific detection of Pneumocystis DNA, by using more sensitive molecular diagnostic assays.

Selection of an initial anti Pneumocystis regimen depends on the severity of the patient illness, other comorbid conditions, and the patient ability to tolerate a specific agent.

6. Product Description

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 is an in vitro diagnostic test, based on real-time PCR technology, for the detection and quantification of Pneumocystis jirovecii specific DNA. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for P. jirovecii DNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of P. jirovecii specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes
The RealStar® Pneumocystis jirovecii PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Four Quantification Standards (QS1 - QS4)
- PCR grade water

Master A and Master B contain all components (buffer, enzymes, primers and probes) to allow PCR mediated amplification and target detection of *P. jirovecii* specific DNA and Internal Control in one reaction setup.

The Quantification Standards contain standardised concentrations of *P. jirovecii* specific DNA. The Quantification Standards can be used individually as positive controls, or together to generate a standard curve, which can be used to determine the concentration of *P. jirovecii* specific DNA in the sample.

The Quantification Standards have the following concentrations:

<table>
<thead>
<tr>
<th>Quantification Standard</th>
<th>Concentration [copies/µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QS1</td>
<td>1.00E+04</td>
</tr>
<tr>
<td>QS2</td>
<td>1.00E+03</td>
</tr>
<tr>
<td>QS3</td>
<td>1.00E+02</td>
</tr>
<tr>
<td>QS4</td>
<td>1.00E+01</td>
</tr>
</tbody>
</table>

6.1 Real-Time PCR Instruments

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® QS/6 plex Platform (QIAGEN)
- CFX96™ Real-Time System (Bio-Rad)
- CFX96™ Deep Well Real-Time System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)
7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.

- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.

- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.

- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.

- Always wear protective disposable powder-free gloves when handling kit components.

- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) 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.

8. Procedure

8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® Pneumocystis jirovecii PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIAasympohony® (QIAGEN)
- NuclSens® easyMag® (bioMérieux)
- MagNa Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate.

The suitability of the nucleic acid extraction procedure for use with RealStar® Pneumocystis jirovecii PCR Kit 1.0 has to be validated by the user.
If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

**CAUTION**

If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

**CAUTION**

The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

### 8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

- If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

<table>
<thead>
<tr>
<th>Number of Reactions (rxns)</th>
<th>1</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master A</td>
<td>5 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Master B</td>
<td>15 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Internal Control</td>
<td>1 µl</td>
<td>12 µl</td>
</tr>
<tr>
<td>Volume Master Mix</td>
<td>21 µl</td>
<td>252 µl</td>
</tr>
</tbody>
</table>

**CAUTION**

If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

<table>
<thead>
<tr>
<th>Number of Reactions (rxns)</th>
<th>1</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master A</td>
<td>5 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Master B</td>
<td>15 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Volume Master Mix</td>
<td>20 µl</td>
<td>240 µl</td>
</tr>
</tbody>
</table>

**CAUTION**

If the IC (Internal Control) was added during the sample preparation procedure, the Master Mix for the controls must be prepared including the IC.
8.3 Reaction Setup

► Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Quantification Standard, Positive or Negative Control).

<table>
<thead>
<tr>
<th>Reaction Setup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master Mix</td>
</tr>
<tr>
<td>Sample or Control</td>
</tr>
<tr>
<td>Total Volume</td>
</tr>
</tbody>
</table>

► Make sure that at least one Positive Control (QS) and one Negative Control is used per run.

► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.

► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

<table>
<thead>
<tr>
<th>Settings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Volume</td>
<td>30 µl</td>
</tr>
<tr>
<td>Ramp Rate</td>
<td>Default</td>
</tr>
<tr>
<td>Passive Reference</td>
<td>ROX™</td>
</tr>
</tbody>
</table>

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

<table>
<thead>
<tr>
<th>Target</th>
<th>Detector Name</th>
<th>Reporter</th>
<th>Quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. jirovecii specific DNA</td>
<td>P. jirovecii</td>
<td>FAM™</td>
<td>(None)</td>
</tr>
<tr>
<td>Internal Control</td>
<td>IC</td>
<td>JOE™</td>
<td>(None)</td>
</tr>
</tbody>
</table>
9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

<table>
<thead>
<tr>
<th>Analysis Mode</th>
<th>Cycle Repeats</th>
<th>Acquisition</th>
<th>Temperature [°C]</th>
<th>Time [min:sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>Hold</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplification</td>
<td>Cycling</td>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Denaturation Hold 1 = 95 02:00
- Amplification Cycling 45 = 95 00:15
- yes 58 00:45
- no 72 00:15

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® Pneumocystis jirovecii PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run (qualitative)

A qualitative diagnostic test run is valid, if the following control conditions are met:

<table>
<thead>
<tr>
<th>Control ID</th>
<th>Detection Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control (QS)</td>
<td>+</td>
</tr>
<tr>
<td>Negative Control</td>
<td>+</td>
</tr>
</tbody>
</table>

10.1.2 Invalid Diagnostic Test Run (qualitative)

A qualitative diagnostic test run is invalid, (i) if the run has not been completed or (ii) if any of the control conditions for a valid diagnostic test run are not met.

In case of an invalid diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.1.3 Valid Diagnostic Test Run (quantitative)

A quantitative diagnostic test run is valid, if all control conditions for a valid qualitative diagnostic test run are met [see chapter 10.1.1 Valid Diagnostic Test Run (qualitative)]. The quantification results are valid if the generated standard curve reaches the following control parameter value:

<table>
<thead>
<tr>
<th>Control Parameter</th>
<th>Valid Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R square (R²)</td>
<td>≥ 0.98</td>
</tr>
</tbody>
</table>

NOTE

Not all real-time PCR instruments display the R square (R²) value. For detailed information, please refer to the user manual of the respective instrument.

10.1.4 Invalid Diagnostic Test Run (quantitative)

A quantitative diagnostic test run is invalid, (i) if the run has not been completed or (ii) if any of the control conditions for a valid quantitative diagnostic test run are not met.

In case of an invalid diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.
10.2 Interpretation of Results

10.2.1 Qualitative Analysis

<table>
<thead>
<tr>
<th>Detection Channel</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FAM™</strong></td>
<td><strong>JOE™</strong></td>
</tr>
<tr>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high *P. jirovecii* DNA load in the sample can lead to a reduced or absent Internal Control signal.

10.2.2 Quantitative Analysis

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 includes four Quantification Standards (QS). In order to generate a standard curve for quantitative analysis, these have to be defined as standards with appropriate concentrations (see chapter 6. Product Description). Using standards of known concentrations a standard curve for quantitative analysis can be generated.

![Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the Amplification Plot [A] and Standard Curve analysis [B]](image)

To determine the fungal load of the original sample, the following formula has to be applied:

\[ \text{Fungal load (Sample)} \ [\text{copies/ml}] = \frac{\text{Volume (Eluate)} [\mu l] \times \text{Fungal load (Eluate)} \ [\text{copies/\mu l}]}{\text{Sample Input} \ [\text{ml}]} \]

**NOTE**

The concentration of the “Sample” is displayed in copies/ml and refers to the concentration in the eluate.
11. Performance Evaluation

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 is defined as the concentration (copies/µl of the eluate) of *P. jirovecii* specific DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified *P. jirovecii* DNA.

<table>
<thead>
<tr>
<th>Input Conc. [copies/µl]</th>
<th>Number of Replicates</th>
<th>Number of Positives</th>
<th>Hit Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.622</td>
<td>16</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>10.000</td>
<td>16</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>3.162</td>
<td>24</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>1.000</td>
<td>24</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>24</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>0.316</td>
<td>24</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>0.100</td>
<td>24</td>
<td>13</td>
<td>54</td>
</tr>
<tr>
<td>0.032</td>
<td>24</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>0.010</td>
<td>24</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>0.003</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The analytical sensitivity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was determined by Probit analysis:

- For the detection of *P. jirovecii* specific DNA, the analytical sensitivity is 0.29 copies/µl [95% confidence interval (CI): 0.19 to 0.55 copies/µl].

11.2 Analytical Specificity

The analytical specificity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant *Pneumocystis* genotypes will be detected.

The analytical specificity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was evaluated by testing a panel of genomic DNA/RNA extracted from pathogens causing similar symptoms as *Pneumocystis jirovecii*.

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis E virus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human herpesvirus 6A
- Human herpesvirus 6B
- Human immunodeficiency virus 1
- Human parvovirus B19
- Influenza A virus H3N2
- Influenza A virus H1N1nv
- Influenza B virus
- Varicella-zoster virus
- Haemophilus influenzae
- Moraxella catarrhalis
- Mycoplasma pneumoniae
- Mycobacterium tuberculosis
- Chlamydia pneumoniae
- Streptococcus pneumoniae
- Streptococcus pyogenes
- Neisseria meningitidis
- Pneumocystis carinii
**11.3 Linear Range**

The linear range of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was evaluated by analyzing a logarithmic dilution series of *Pneumocystis jirovecii* specific DNA using concentrations ranging from $10^8$ copies/µl to $10^1$ copies/µl. At least eight replicates per dilution were analysed.

![Amplification curves](image)

The linear range of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was determined to be $10^1$ copies/µl to $10^8$ copies/µl.

**11.4 Precision**

Precision of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analyses.

Variability data are expressed in terms of standard deviation and coefficient of variation. The data are based on quantification analysis of defined concentrations of *P. jirovecii* specific DNA and on threshold cycle ($C_T$) value in terms of the Internal Control. At least six replicates per sample were analysed for intra-assay, inter-assay and inter-lot variability.

<table>
<thead>
<tr>
<th></th>
<th>Average Conc..tokens/µl</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay Variability</td>
<td>3.72</td>
<td>0.01</td>
<td>0.26</td>
</tr>
<tr>
<td>Inter-Assay Variability</td>
<td>3.76</td>
<td>0.05</td>
<td>1.35</td>
</tr>
<tr>
<td>Inter-Lot Variability</td>
<td>3.74</td>
<td>0.02</td>
<td>0.62</td>
</tr>
<tr>
<td>Total Variability</td>
<td>3.76</td>
<td>0.04</td>
<td>1.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Internal Control</th>
<th>Average Threshold Cycle ($C_T$)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay Variability</td>
<td>26.20</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>Inter-Assay Variability</td>
<td>26.18</td>
<td>0.07</td>
<td>0.29</td>
</tr>
<tr>
<td>Inter-Lot Variability</td>
<td>26.16</td>
<td>0.08</td>
<td>0.32</td>
</tr>
<tr>
<td>Total Variability</td>
<td>26.16</td>
<td>0.08</td>
<td>0.31</td>
</tr>
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</table>
12. Limitations

- Strict compliance with the instructions 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 \textit{in vitro} diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause underquantification, false negative or invalid results.
- Potential mutations within the target regions of the P. jirovecii genome covered by the primers and/or probes used in the kit may result in underquantification and/or failure to detect the presence of the pathogen.
- As with any diagnostic test, results of the RealStar\textsuperscript{®} Pneumocystis jirovecii PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar\textsuperscript{®} Pneumocystis jirovecii PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For technical advice, please contact our Technical Support:

- e-mail: support@altona-diagnostics.com
- phone: +49-(0)40-5480676-0

15. Literature


16. Trademarks and Disclaimers

RealStar\textsuperscript{®} (altona Diagnostics); Mx 3005P\textsuperscript{™} (Stratagene); ABI Prism\textsuperscript{®} (Applied Biosystems); Rotor-Gene\textsuperscript{®}; QIAamp\textsuperscript{®}, QIAasymp\textsuperscript{®} (QIAGEN); CFX96\textsuperscript{™} (Bio-Rad); Maxwell\textsuperscript{®} (Promega); NucliSENS\textsuperscript{®}, easyMag\textsuperscript{®} (bioMérieux); VERSANT\textsuperscript{®} (Siemens Healthcare); LightCycler\textsuperscript{®} (Roche); FAM\textsuperscript{™}, JOE\textsuperscript{™}, ROX\textsuperscript{™} (Life Technologies); Cy\textsuperscript{®} (GE Healthcare).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar\textsuperscript{®} Pneumocystis jirovecii PCR Kit 1.0 is a CE-marked diagnostic kit according to the European \textit{in vitro} diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved. Not available in all countries.

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17. **Explanation of Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVD</td>
<td><em>In vitro</em> diagnostic medical device</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
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<tr>
<td>CAP</td>
<td>Cap color</td>
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<tr>
<td>REF</td>
<td>Product number</td>
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<tr>
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<td>Content</td>
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<tr>
<td>NUM</td>
<td>Number</td>
</tr>
<tr>
<td>COMP</td>
<td>Component</td>
</tr>
<tr>
<td>GTIN</td>
<td>Global trade identification number</td>
</tr>
<tr>
<td>🔧</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>🔜</td>
<td>Contains sufficient for “n” tests/reactions (rxns)</td>
</tr>
<tr>
<td>🔄</td>
<td>Temperature limit</td>
</tr>
<tr>
<td>🕒</td>
<td>Use-by date</td>
</tr>
<tr>
<td>🏭</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>🚸</td>
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<tr>
<td>📄</td>
<td>Note</td>
</tr>
<tr>
<td>🗏</td>
<td>Version</td>
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</tbody>
</table>

**Notes:**
always a drop ahead.