Atypical Pneumonia-1 assay

For the detection of *Pneumocystis jirovecii*, *Toxoplasma gondii* and *Legionella pneumophila* using the BD MAX™ system

Instructions for use

(Version 3.0 – Februar 2018)
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Introduction

Atypical pneumonia (AP) comprise community and healthcare-associated infections caused by pathogens, which necessitate distinct antibiotic therapy and are hardly detectable by culture-based methods.

This protocol describes the system settings and setup protocols for running the AP-1 panel to detect three atypical pneumonia pathogens using the BD MAX™ system (assay target genes):

1. *Pneumocystis jirovecii* (β-tubulin gene)
2. *Legionella pneumophila* (mip gene)
3. *Toxoplasma gondii* (B1 gene)
4. Internal control of DNA-extraction (β-actin gene)

The qPCR has been validated for respiratory specimens such as bronchoalveolar lavage, endotracheal aspirate and sputum sample.

Contact information

For information regarding ordering dried snap-in tubes for the AP-1 assay:

info@biolegio.com

For information regarding to the protocol:

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1. **Protocol**

This protocol describes the assay settings required for running the AP-1 assay on the BD MAX™ system. The AP-1 snap-in assay contains primers and probes for the detection of *Pneumocystis jirovecii*, *Toxoplasma gondii* and *Legionella pneumophila*. Additionally, the assay provides primers and probe for β-actin of eukaryotic cells used as internal control for the DNA extraction and PCR performance.

1.1 **Materials needed**

- BD MAX system
- BD MAX™ ExK DNA-2 Extraction Kit (BD cat.no: 442820)
- BD MMK Mastermix without SPC (BD cat.no: 442848)
- BD MAX™ PCR Cartridges (BD cat.no: 437519)
- Dried snap-ins AP-1 (Biolegio cat no: BDT-14007-24)
- Vortex Mixer
- Centrifuge
- Micropipettes
- Safeseal Filtertips
- Disposable gloves

1.2 **Run settings**

The assay is performed on the BD MAX with use of the BD MMK in combination with the ExK DNA-2 Kit for the extraction.

Create a full process assay in the test editor named “AP-1 assay” and use the following parameters:
Edit the test steps using the following settings:
1.3. Sample preparation

Respiratory specimens: bronchoalveolar lavage, endotracheal aspirate or sputum samples.

- Viscous sputum-samples will be pre-treated with sputasol or trypsin (if necessary) for liquefaction.
- Liquid samples (1 ml) need to be centrifuged at a speed of 16000 g for 5 minutes, then remove 700 µl of the supernatant and resuspend the rest of the sample.
- Extraction will be done with the “BD MAX™ ExK DNA-2 Extraction Kit.”

Transfer 200 µl of the sample into a BD MAX™ DNA Sample Buffer Tube and close the tube with a blue septum cap. Ensure complete mixing by vortexing the sample.

1.4. Setting up the experiment

a. Create a worklist on the BD MAX instrument using the AP-1 assay (created in step 1.2) and label the lanes with the sample names.

b. Load the prepared Sample Buffer Tubes into their corresponding position in the BD MAX racks.
c. Load the BD MAX racks with the corresponding Unitized Reagent Strip.

d. Snap-in the BD Extraction tubes (position 1), MMK tubes (position 2) and AP-1 tubes (position 3) into the Reagent Strip.

e. Load the racks and cartridges into the BD MAX and Start Run

2. Result interpretation

2.1 For a run to be valid

- No BD MAX System failures.
- Negative Control (optional) has a Cq value of -1 for all channels
- Positive control (optional) has a Cq value for channel 475/520, 530/565, 630/665 and 680/715

2.2 Interpretation if run is valid

- A Cq value of -1 indicates a negative result
- A Cq value for either of the targets indicates a positive result for the corresponding target.
- The β-actin extraction control (channel 680/715) should always give a Cq value. A negative Cq value for the β-actin indicates inhibition and therefore the test of this sample should be repeated.
- All curves need to be visually checked for right interpretation.

Attention!
In case of positive results obtained for two or more targets of the panel, the fluorescence intensity of all positive signals needs to be checked. If the signal intensity of the color channels differs considerably (≥ 1:10, e.g. “3000” in one channel and “300” in the other), than a artificial cross-talk cannot be excluded. Thus, the result of the channel with lower intensity is doubtful and needs to be confirmed by another specific PCR test.
3. Validation

3.1 Targets

3.1.1. *Pneumocystis jirovecii*: β-tubulin gene (GenBank Acc. No.: AF17964)

3.1.2. *Toxoplasma gondii*: B1 gene (GenBank Acc. No.: AF179871)

3.1.3. *Legionella pneumophila*: mip gene (GenBank Acc. No.: AF095230)

3.2. Sensitivity / Specificity / Analytical Sensitivity

- The sensitivity and specificity was determined using XX defined positive and YY defined negative samples, which were unequivocally assessed positive and negative by 2 independent other PCRs, respectively.
- The analytical sensitivity was determined by log-step dilutions of plasmids carrying the respective DNA target sequences.
- All PCRs have been used in our clinical microbiological diagnostic laboratory for the last 8 years under accreditation having passed constantly external inter-laboratory comparison programmes (twice a year).

<table>
<thead>
<tr>
<th>Target</th>
<th>analytical sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pneumocystis jirovecii</em></td>
<td>$10^4$ copies/ ml</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>$10^4$ copies/ ml</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>$10^2$ copies/ ml</td>
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</tbody>
</table>

Disclaimer:

MvP-Institut is not responsible for the results on the AP-1 assay on the BD MAX™ system. Using the „open protocol“, the respective laboratory itself is responsible for the validation of the assay.