AmpliSens® *Mycoplasma hominis*-EPh

PCR kit

Instruction Manual
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1. INTENDED USE

AmpliSens® *Mycoplasma hominis*-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycoplasma hominis* in the clinical material (cervical or urethral scrapes (swabs), urine sediment, secret of the prostate gland) by means of detection of the amplified products in agarose gel.

⚠️ The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

*Mycoplasma hominis* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Mycoplasma hominis* primers. After PCR the amplified product is detected in agarose gel. AmpliSens® *Mycoplasma hominis*-EPh PCR kit is a qualitative test and contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual specimen and to identify possible reaction inhibition. AmpliSens® *Mycoplasma hominis*-EPh PCR kit uses “hot-start”, that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Melting of wax and mix of reaction components occur only at 95 °C, which greatly diminish frequency of nonspecifically primed reactions.

3. CONTENT

AmpliSens® *Mycoplasma hominis*-EPh PCR kit is produced in 2 forms:

AmpliSens® *Mycoplasma hominis*-EPh PCR kit variant 100 R (tubes 0.5 ml),

[REF] B3-100-R0,5-CE.

AmpliSens® *Mycoplasma hominis*-EPh PCR kit variant 100 R (tubes 0.2 ml),

[REF] B3-100-R0,2-CE.
AmpliSens® Mycoplasma hominis-EPh PCR kit variant 100 R includes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Volume (ml)</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-mix-1 R <em>Mycoplasma hominis</em> ready-to-use single-dose test tubes (under wax)</td>
<td>colorless clear liquid</td>
<td>0.005</td>
<td>110 tubes of 0.5 or 0.2 ml</td>
</tr>
<tr>
<td>PCR-mix-2 blue</td>
<td>blue clear liquid</td>
<td>1.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>Mineral oil for PCR</td>
<td>colorless viscous liquid</td>
<td>4.0</td>
<td>1 vial</td>
</tr>
<tr>
<td><strong>Positive Control DNA <em>Mycoplasma hominis</em> (C+)</strong></td>
<td>colorless clear liquid</td>
<td>0.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>DNA-buffer</td>
<td>colorless clear liquid</td>
<td>0.5</td>
<td>1 tube</td>
</tr>
<tr>
<td>Negative Control (C-)*</td>
<td>colorless clear liquid</td>
<td>1.2</td>
<td>1 tube</td>
</tr>
<tr>
<td><strong>Internal Control complex (ICc)</strong>**</td>
<td>colorless clear liquid</td>
<td>1.0</td>
<td>1 tube</td>
</tr>
</tbody>
</table>

* must be used in the extraction procedure as Negative Control of Extraction.
** add 10 µl of Internal Control complex during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, REF K1-12-100-CE or DNA-sorb-B, REF K1-2-100-CE protocols).

AmpliSens® Mycoplasma hominis-EPh PCR kit variant 100 R is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Agarose gel detection kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max 16,000 x g).
- PCR box or Biological cabinet.
- Vacuum aspirator with flask for removing supernatant.
- Tube racks.
- 1.5 ml polypropylene sterile tubes.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤–16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.
• Thermostat for tubes with controlled temperature and capable of incubating at 25°C to 100 °C.
• Personal thermocyclers (for example, Terzik (DNA-Technology, Russia), Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygen Scientific, USA)).

5. GENERAL PRECAUTIONS.
The user should always pay attention to the following:
• Use sterile RNase-free pipette tips with aerosol filters and use new tip for every procedure.
• Store and handle amplicons away from all other reagents.
• Thaw all components thoroughly at room temperature before starting detection.
• When thawed, mix the components and centrifuge briefly.
• Use protective gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
• Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
• Do not use a kit after its expiration date.
• Dispose of all samples and unused reagents in compliance with local authorities requirements.
• Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
• Clean and disinfect all sample or reagent spills with 0.5 % sodium hypochlorite solutions or other suitable disinfectant.
• Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, seek medical attention
• Material Safety Data Sheets (MSDS) are available on request.
• Use of this product should be limited to personnel trained in the techniques of DNA amplification.
• The laboratory process must be unidirectional; it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area where you carried out the previous step.

Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining of biological materials sample for PCR-analysis, transportation and storage are described in manufacturer’s handbook [2]. It is recommended that this handbook is read before starting of work.
AmpliSens® *Mycoplasma hominis*-EPh PCR kit is intended for PCR-analysis of DNA extracted with DNA extraction kits from:

- *Cervical or urethral scrapes (swabs).*
- *Urine sediment (use the first portion of the morning urine specimen).*
- *Secret of the prostate gland.*

7. WORKING CONDITIONS

*AmpliSens® Mycoplasma hominis*-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

7.1. DNA Extraction

It’s recommended to use following nucleic acid extraction kits:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- DNA-sorb-B (for secret of the prostate gland), **REF** K1-2-100-CE.

⚠️ Please carry out the DNA extraction in compliance with the manufacturer protocol.

8.2. Preparing the PCR.

Total reaction volume is **25 µl**, volume of DNA sample is **10 µl**.

8.2.1. Preparing tubes for PCR.

1. Prepare the required number of the tubes prepared as describes above or tubes with **PCR-mix-1-R Mycoplasma hominis** with wax for amplification of clinical and control samples DNA.
2. Add **10 µl** of **PCR-mix-2 blue** to the surface of wax layer of each tube ensuring that it does not fall under the wax and mix with reagents in the tube.
3. Add above **1 drop** of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.
4. Under or immediately above the oil level, add **10 µl** of **DNA samples**, obtained from clinical or control samples at the stage of DNA extraction. Use tips with aerosol filter.
5. Carry out the **control amplification reactions**:
   - NCA – Add **10 µl** of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
   - C+ – Add **10 µl** of **Positive Control DNA Mycoplasma hominis** to the tube for Positive Control of Amplification.

8.2.2. Amplification.

Run the following program on the thermocycler (see table 1). When the temperature will reach 95 °C (pause regimen), insert the tubes into the cells of amplifier and press the
button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 s) before their insertion in a thermocycler.

Table 1

Programming of the thermocyclers for *Mycoplasma hominis* DNA amplification.

<table>
<thead>
<tr>
<th>Step</th>
<th>Thermocyclers with active temperature adjustment:</th>
<th>Thermocyclers with block temperature adjustment:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Terzik (DNA-Technology)</td>
<td>GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)</td>
</tr>
<tr>
<td></td>
<td>Thermocyclers with active temperature adjustment:</td>
<td>Thermocyclers with block temperature adjustment:</td>
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<tr>
<td></td>
<td>Terzik (DNA-Technology)</td>
<td>GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)</td>
</tr>
<tr>
<td>0</td>
<td>95 °C pause</td>
<td>95 °C pause</td>
</tr>
<tr>
<td>1</td>
<td>95 °C 5 min 1</td>
<td>95 °C 5 min 1</td>
</tr>
<tr>
<td>2</td>
<td>95 °C 10 s 42</td>
<td>95 °C 15 s 42</td>
</tr>
<tr>
<td>3</td>
<td>72 °C 1 min 1</td>
<td>72 °C 1 min 1</td>
</tr>
<tr>
<td>4</td>
<td>4 °C storage</td>
<td>4 °C storage</td>
</tr>
</tbody>
</table>

Amplification in the thermocycler with block temperature adjustment lasts 2 h 30 min, in the thermocycler with active temperature adjustment – 1 h 50 min.

After the reaction is finished the PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel. The amplified samples can be stored for 16 h at room temperature, for 1 week at 2–8 °C and for a long time at minus 16 °C (be sure to warm the samples to room temperature before running electrophoresis).

**9. DATA ANALYSIS**

It’s recommended to use the following detection agarose kit:

- EPh variant 200, [REF K5-200-CE](#).

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%). The length of specific amplified DNA fragments is:

- *Mycoplasma hominis* – 330 bp
- Internal Control complex – 550 bp

⚠️ Put the protective mask or use the glass filter while watching and photographing the gel.
9.1. Results interpretation

Table 2

<table>
<thead>
<tr>
<th>Control</th>
<th>Which step of test is controlled</th>
<th>Specific bands in the agarose gel</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-</td>
<td>DNA extraction</td>
<td>330 bp</td>
<td>No</td>
</tr>
<tr>
<td>NCA</td>
<td>Amplification</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>C+</td>
<td>Amplification</td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

- The sample is considered positive for *Mycoplasma hominis* DNA if the band of 330 bp is present in agarose gel regardless of the ICc band presence. The band of ICc (550 bp) could be absent in the samples with high concentration of *Mycoplasma hominis* DNA.

- The sample is considered negative for *Mycoplasma hominis* DNA if the band of 330 bp is absent and the band of 550 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp.

10. TROUBLESHOOTING

If analysis results are not obtained as per the following examples:

- If the results of control samples do not correspond to the listed above (Table 2), then the tests should be repeated.

- If in lanes none of bands of 330 and 550 nucleotide pairs is observed for any clinical samples, the test results of this sample is irrelevant and the analysis should be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.

- If in lines nonspecific bands are present at different levels, it may be caused by lack of “hot start” or false temperature regimen in the thermocycler.

- If in lanes corresponding to negative controls (NCA, C–) specific band of 330 bp appears it means that reagents or samples contamination has taken place. In such cases analysis results must be considered as irrelevant. Test analysis should be repeated and measures for detecting contamination source must be undertaken.

If you have any further questions or encounter problems, please contact our Authorized Representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Mycoplasma hominis*-EPh PCR kit should be transported at 2–8 °C for no longer than 5 days.
12. STABILITY AND STORAGE
All components of AmpliSens® Mycoplasma hominis-EPh PCR kit should be stored at 2–8 °C when not in use. They also must be stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

13. SPECIFICATIONS
Analytical Sensitivity of AmpliSens® Mycoplasma hominis-EPh PCR kit is no less than $5 \times 10^3$ colony forming units per 1 ml of a sample (CFU/ml).

⚠️ The claimed analytical features of AmpliSens® Mycoplasma hominis-EPh PCR kit are guaranteed only when additional kits of reagents, DNA-sorb-AM or DNA-sorb-B, (for secret of the prostate gland) and EPh are used.

13.2. Specificity.
Specificity of AmpliSens® Mycoplasma hominis-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

14. REFERENCES.

15. QUALITY CONTROL.
In compliance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of AmpliSens® Mycoplasma hominis-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.
16. KEY TO SYMBOLS USED

[Image of symbols]

- **REF** Catalogue number
- **LOT** Batch code
- **IVD** In vitro diagnostic medical device
- **VER** Version
- **NCA** Negative control of amplification
- **C−** Negative control of extraction
- **C+** Positive control of amplification
- **ICc** Internal Control complex
- **Authorised representative in the European Community**
### List of Changes Made in the Instruction Manual

<table>
<thead>
<tr>
<th>VER</th>
<th>Location of changes</th>
<th>Essence of changes</th>
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<tbody>
<tr>
<td>12.11.10</td>
<td>Through the text</td>
<td>Records about PCR kit variant 200 are deleted</td>
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<tr>
<td></td>
<td>Cover page</td>
<td>The phrase “For Professional Use Only” was added</td>
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<tr>
<td></td>
<td>Intended use</td>
<td>The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added.</td>
</tr>
<tr>
<td>25.12.10</td>
<td>Content</td>
<td>New sections “Working Conditions” and “Transportation” were added</td>
</tr>
<tr>
<td>KM</td>
<td>Stability and Storage</td>
<td>The “Explanation of Symbols” section was renamed to “Key to Symbols Used”</td>
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<tr>
<td></td>
<td>Key to Symbols Used</td>
<td>The information about the shelf life of open reagents was added</td>
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<tr>
<td>24.06.11</td>
<td>Cover page, text</td>
<td>The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”</td>
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