AmpliSens® *Chlamydia trachomatis*-FRT

PCR kit

Instruction Manual
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1. INTENDED USE

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Chlamydia trachomatis* DNA in the clinical material (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; urine; and prostate gland secretion) using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

*Chlamydia trachomatis* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Chlamydia trachomatis* primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. In variant FRT, “hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction components mix only at 95 °C. In variant FRT-100 F, “hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is produced in 2 forms:

- AmpliSens® *Chlamydia trachomatis*-FRT PCR kit variant FRT, REF R-B1(RG)-CE.
- AmpliSens® *Chlamydia trachomatis*-FRT PCR kit variant FRT-100 F REF R-B1-F(RG,iQ)-CE.
AmpliSens® *Chlamydia trachomatis*-FRT PCR kit, variant FRT includes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Volume, ml</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-mix-1-FL <em>Chlamydia trachomatis</em> ready-to-use single-dose test tubes <em>(under wax)</em></td>
<td>colorless clear liquid</td>
<td>0.01</td>
<td>110 tubes of 0.2 ml</td>
</tr>
<tr>
<td>PCR-mix-2-FL-red</td>
<td>red clear liquid</td>
<td>1.1</td>
<td>1 tube</td>
</tr>
<tr>
<td>Positive Control complex (C+)</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>Internal Control-FL (IC)**</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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM REF K1-12-100-CE protocol).

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is intended for 110 reactions, including controls.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit, variant FRT-100 F includes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Volume, ml</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-mix-1-FL <em>Chlamydia trachomatis</em></td>
<td>colorless clear liquid</td>
<td>1.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>PCR-mix-2-FRT</td>
<td>colorless clear liquid</td>
<td>0.3</td>
<td>2 tubes</td>
</tr>
<tr>
<td>Polymerase (TaqF)</td>
<td>colorless clear liquid</td>
<td>0.03</td>
<td>2 tubes</td>
</tr>
<tr>
<td>Positive Control complex (C+)</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>Internal Control-FL (IC)**</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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM REF K1-12-100-CE protocol).

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is intended for 110 reactions, including controls.
4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
  a) 0.2-ml thin-walled PCR tubes domed caps if a plate-type instrument is used;
  b) 0.2-ml thin-walled PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and 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 specimens and unused reagents in accordance with local regulations.
• Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
• Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
• Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
• Safety Data Sheets (SDS) are available on request.
• Use of this product should be limited to personnel trained in DNA amplification techniques.
• Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in the manufacturer’s handbook [1]. It is recommended that this handbook is read before starting work. AmpliSens® Chlamydia trachomatis-FRT PCR kit is intended for analysis of the DNA extracted with the use of DNA extraction kits from the clinical material (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; urine (a sediment of the first portion of the morning specimen), prostate gland secretion).

7. WORKING CONDITIONS

AmpliSens® Chlamydia trachomatis-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

It is recommended that the following nucleic acid extraction kits are used:
• DNA-sorb-AM, [REF] K1-12-100-CE.
• For other nucleic acid extraction kits see Guidelines [2].

The DNA extraction of each test sample is carried out in the presence Internal Control-FL (IC).
8. Extract the DNA according to the manufacturer's protocol.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis.
Use disposable filter tips for adding reagents, DNA and control samples into tubes.

Variant FRT

The total reaction volume is 30 µl, the volume of DNA sample is 10 µl.
1. Collect the required number of the tubes with PCR-mix-1-FL Chlamydia trachomatis and wax for amplification of DNA from clinical and control samples.
2. Add 10 µl of PCR-mix-2-FL-red to the surface of the wax layer into each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-FL Chlamydia trachomatis.

Variant FRT-100 F

The total reaction volume is 25 µl, the volume of DNA sample is 10 µl.
1. Thaw the PCR-mix-2-FRT tube. Vortex the tubes with PCR-mix-1-FL Chlamydia trachomatis, PCR-mix-2-FRT, and polymerase (TaqF) and then centrifuge briefly.

Take the required number of strip or unstrip tubes for amplification of DNA from clinical and control samples.
2. For N reactions (including 2 controls), add to a new tube:
   10*(N+1) µl of PCR-mix-1-FL Chlamydia trachomatis,
   5.0*(N+1) µl of PCR-mix-2-FRT,
   0.5*(N+1) µl of polymerase (TaqF).

Vortex the tube and then centrifuge briefly. Transfer 15 µl of the prepared mixture to each tube.

Steps 3 and 4 are required in both variants.
3. Using filter tips, add 10 µl of DNA samples obtained at the stage of DNA extraction.
4. Perform control amplification reactions:

   NCA – Add 10 µl of DNA-buffer to the tube labeled NCA (Negative Control of Amplification).

   C+ – Add 10 µl of Positive Control complex (C+) (to the tube labeled C+ (Positive Control of Amplification).

   C– – Add 10 µl of the sample extracted from the Negative Control (C–) to the tube labeled C– (Negative Control of Extraction).
8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Rotor-type Instruments</th>
<th>Plate-type Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature, °C</td>
<td>Time</td>
</tr>
<tr>
<td>1</td>
<td>95</td>
<td>15 min</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>5 s</td>
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<tr>
<td></td>
<td>60</td>
<td>20 s</td>
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<td></td>
<td>72</td>
<td>15 s</td>
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<tr>
<td>3</td>
<td>95</td>
<td>5 s</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20 s</td>
</tr>
<tr>
<td></td>
<td>fluorescent signal detection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>15 s</td>
</tr>
</tbody>
</table>

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (if other tests are performed simultaneously, the detection is assigned in other used channels).

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].

3. Insert tubes into the reaction module of the device.

4. Run the amplification program with fluorescence detection.

5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

- The signal of the *Chlamydia trachomatis* DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the IC DNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- *Chlamydia trachomatis* DNA is **detected** if *Ct* value is determined in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the

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1 For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
2 For example, iCycler, iQ5, Mx3000P, Mx3000, or equivalent.
threshold line in the area of typical exponential growth of fluorescence.

- *Chlamydia trachomatis* DNA is **not detected** if the *Ct* value is not determined (absent) in the channels for FAM fluorophores, whereas the *Ct* value determined in the channel for the JOE fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.

- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for FAM fluorophores, whereas the *Ct* value in the channel for the JOE fluorophore is not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated for the corresponding clinical sample.

⚠️ Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2].

**The result of analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 2).**

<table>
<thead>
<tr>
<th>Control</th>
<th>Stage for control</th>
<th>Ct value in the channel for fluorophore</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>C−</td>
<td>DNA extraction</td>
<td>Absent</td>
</tr>
<tr>
<td>NCA</td>
<td>PCR</td>
<td>Absent</td>
</tr>
<tr>
<td>C+</td>
<td>PCR</td>
<td>&lt; boundary value</td>
</tr>
</tbody>
</table>

### 10. TROUBLESHOOTING

1. If the *Ct* value of the Positive Control of Amplification (C+) in the channel for the FAM fluorophore is absent or greater than the boundary value, the amplification should be repeated for all samples in which *Chlamydia trachomatis* cDNA was not detected.

2. If the *Ct* value is determined for the Negative Control of Extraction (C−) and/or Negative Control of Amplification (NCA) in the channel for the FAM fluorophore, repeat PCR analysis for all samples in which *Chlamydia trachomatis* DNA was detected starting from the DNA extraction stage.

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

### 11. TRANSPORTATION

**AmpliSens® Chlamydia trachomatis-FRT** PCR kit should be transported at 2–8 °C for no longer than 5 days.
12. STABILITY AND STORAGE

All components of the AmpliSens® 
*Chlamydia trachomatis*-FRT PCR kit are to be stored at 2–8 °C when not in use (except for Polymerase (TaqF) and PCR-mix-2-FRT). All components of the AmpliSens® 
*Chlamydia trachomatis*-FRT PCR kit are 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.

⚠️ Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C.

⚠️ PCR-mix-1-FL *Chlamydia trachomatis* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of AmpliSens® 
*Chlamydia trachomatis*-FRT PCR kit is specified in the table below.

<table>
<thead>
<tr>
<th>Clinical material</th>
<th>Transport medium</th>
<th>DNA extraction kit</th>
<th>Analytical sensitivity, GE/ml(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urogenital swabs</td>
<td>Transport Medium for Swabs [REF] 987-CE or Transport Medium with Mucolytic Agent [REF] 953-CE</td>
<td>DNA-sorb-AM</td>
<td>5 x 10(^2)</td>
</tr>
<tr>
<td>Urine (pretreatment is required)</td>
<td>–</td>
<td>DNA-sorb-AM</td>
<td>1 x 10(^3)</td>
</tr>
</tbody>
</table>

13.2. Specificity

The analytical specificity of AmpliSens® 
*Chlamydia trachomatis*-FRT PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Nonspecific responses were absent during examination of human DNA as well as a DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Candida albicans, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Neisseria flava, Neisseria subflava, Neisseria sicca,*

\(^3\) Genome equivalents (GE) of the microorganism per 1 ml of a clinical material placed into the specified transport medium.
Neisseria mucosa, Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV type 1 and 2, CMV, and HPV.

The clinical specificity of AmpliSens® Chlamydia trachomatis-FRT PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES
2. Guidelines “Real-Time PCR Detection of STIs and Other Reproductive Tract Infections”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL
In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the AmpliSens® Chlamydia trachomatis-FRT PCR kit has been tested against predetermined specifications to ensure consistent product quality.
### 16. KEY TO SYMBOLS USED

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td><img src="image" alt="Ref" /></td>
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<tr>
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<td>Caution</td>
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<tr>
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<td>Batch code</td>
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<tr>
<td><img src="image" alt="Sufficient" /></td>
<td>Sufficient for</td>
</tr>
<tr>
<td><img src="image" alt="IVD" /></td>
<td><em>In vitro</em> diagnostic medical device</td>
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</tr>
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<td>Version</td>
</tr>
<tr>
<td><img src="image" alt="Consult" /></td>
<td>Consult instructions for use</td>
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<tr>
<td><img src="image" alt="Temperature Limitation" /></td>
<td>Temperature limitation</td>
</tr>
<tr>
<td><img src="image" alt="Keep Away From Sunlight" /></td>
<td>Keep away from sunlight</td>
</tr>
<tr>
<td><img src="image" alt="Manufacturer" /></td>
<td>Manufacturer</td>
</tr>
<tr>
<td><img src="image" alt="NCA" /></td>
<td>Negative control of amplification</td>
</tr>
<tr>
<td><img src="image" alt="Date of Manufacture" /></td>
<td>Date of manufacture</td>
</tr>
<tr>
<td><img src="image" alt="C-" /></td>
<td>Negative control of extraction</td>
</tr>
<tr>
<td><img src="image" alt="Authorised Representative in the European Community" /></td>
<td>Authorised representative in the European Community</td>
</tr>
<tr>
<td><img src="image" alt="C+" /></td>
<td>Positive control of amplification</td>
</tr>
<tr>
<td><img src="image" alt="IC" /></td>
<td>Internal control</td>
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## List of Changes Made in the Instruction Manual

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<thead>
<tr>
<th>VER</th>
<th>Location of changes</th>
<th>Essence of changes</th>
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</thead>
<tbody>
<tr>
<td>23.06.11 LA</td>
<td>Cover page, text</td>
<td>The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”</td>
</tr>
<tr>
<td>24.09.15 PM</td>
<td>Text</td>
<td>Corrections according to the template</td>
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<td>1. Intended use</td>
<td>The clinical material was specified</td>
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<td></td>
<td>6. Sampling and handling</td>
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<td>3. Content, Footer</td>
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<tr>
<td></td>
<td>13.1. Sensitivity</td>
<td>The catalogue numbers for the transport media were added</td>
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