AmpliSens® Treponema pallidum-FRT PCR kit
Instruction Manual
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1. INTENDED USE

AmpliSens® Treponema pallidum-FRT PCR kit is an in vitro nucleic acid amplification test for qualitative detection of Treponema pallidum DNA in the clinical material (urogenital, rectal, and oral swabs; blister exudate; and discharge of erosive-ulcer lesions of human skin and mucous membranes) 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

Treponema pallidum detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific Treponema pallidum 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® Treponema pallidum-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® Treponema pallidum-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 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 using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® Treponema pallidum-FRT PCR kit is produced in 2 forms:

AmpliSens® Treponema pallidum-FRT PCR kit variant FRT, [REF] R-B20(RG)-CE.

AmpliSens® Treponema pallidum-FRT PCR kit variant FRT-100 F, [REF] R-B20-F(RG,iQ)-CE.

AmpliSens® Treponema pallidum-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 Treponema pallidum</td>
<td>clear liquid from colorless to light lilac colour</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 (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, REF K1-12-100-CE protocol).

AmpliSens® Treponema pallidum-FRT PCR kit is intended for 110 reactions (including controls).

AmpliSens® Treponema pallidum-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 Treponema pallidum</td>
<td>clear liquid from colorless to light lilac colour</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>
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<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 (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, REF K1-12-100-CE protocol).

AmpliSens® Treponema pallidum-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 a 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), or equivalent).
- Disposable polypropylene tubes when working with PCR kit variant FRT-100 F:
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed caps if a plate-type instrument is used;
  b) thin-walled 0.2-ml 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 for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters 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® *Treponema pallidum*-FRT PCR kit is intended for analysis of DNA extracted by DNA extraction kits from the clinical material (urogenital, rectal, and oral swabs; blister exudate; and discharge of erosive-ulcer lesions of human skin and mucous membranes).

7. WORKING CONDITIONS

AmpliSens® *Treponema pallidum*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- 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 of Internal Control-FL (IC).

In the extraction procedure it is necessary to carry out the control reactions as follows:

C–  Add 100 µl of Negative Control (C–) to the tube labeled C–.

⚠️ Extract DNA according to the manufacturer’s protocols.

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. Prepare the required number of tubes with PCR-mix-1-FL *Treponema pallidum* 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 *Treponema pallidum*.

Variant FRT-100F

The total reaction volume is 25 µl, the volume of DNA sample is 10 µl.

1. Thaw the tube with PCR-mix-2-FRT. Vortex the tubes with PCR-mix-1-FL *Treponema pallidum*, PCR-mix-2-FRT, and polymerase (TaqF) and sediment the drops by short centrifugation (1-2 s).

   Take the required quantity of tubes/ strips for amplification of DNA from clinical and control samples.

2. For N reactions (including 2 controls of amplification) add to a new tube:
   
   - 10-(N+1) µl of PCR-mix-1-FL *Treponema pallidum*,
   - 5.0-(N+1) µl of PCR-mix-2-FRT,
   - 0.5-(N+1) µl of polymerase (TaqF).

   Mix the prepared mixture and sediment the drops by short centrifugation (1-2 s).

   Transfer 15 µl of the prepared mixture into each tube.

Steps 3 and 4 are required out in both variants.

3. Add 10 µl of DNA obtained at the DNA extraction stage into the prepared tubes.

4. Carry out the 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 sample extracted from Negative Control (C−) reagent to the tube labeled C− (Negative Control of Extraction).

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:
Fluorescent signal is detected in the channels for the FAM and JOE fluorophores. Other channels are enabled if several tests are simultaneously carried out in a single run.

2. Adjust the fluorescence channel sensitivity according to Important Product Information Bulletin.

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 *Treponema pallidum* DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the IC 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:

- *Treponema pallidum* DNA is detected in a sample if the \( Ct \) value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of exponential growth of fluorescence.

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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.
- *Treponema pallidum* DNA is **not detected** if its *Ct* value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channel for the FAM fluorophore, whereas the *Ct* value determined in the results grid in the channel for the JOE fluorophore does not exceed the specified boundary value.

- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the FAM fluorophore, whereas the *Ct* value in the channel for the JOE fluorophore is not determined (absent) or exceeds specified boundary value. In such case, PCR should be repeated for this sample.

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

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

Table 2

<table>
<thead>
<tr>
<th>Control</th>
<th>Stage for control</th>
<th>Ct value in the channel for fluorophore</th>
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<td></td>
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<td>C–</td>
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<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**

Results of analysis are not taken into account in the following cases:

1. The *Ct* value determined for the Positive Control of amplification (C+) in the channel for the FAM fluorophore is greater than the specified boundary value or absent. The amplification should be repeated for all the samples in which the *Treponema pallidum* DNA was not detected.

2. The *Ct* value is determined for the Negative Control of Extraction (C–) and/or the Negative Control of Amplification (NCA) in the channel for the FAM fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Treponema pallidum* DNA was detected.

If you have any further questions or if you encounter problems, please contact our Authorized Representative in the European Community.
11. TRANSPORTATION
AmpliSens® *Treponema pallidum*-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® *Treponema pallidum*-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® *Treponema pallidum*-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 temperature from minus 24 to minus 16 °C.
- PCR-mix-1-FL *Treponema pallidum* is to be kept away from light.

13. SPECIFICATIONS
13.1. Sensitivity

<table>
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<tr>
<th>Clinical material</th>
<th>Transport medium</th>
<th>Nucleic acid extraction kit</th>
<th>Sensitivity, GE/ml $^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urogenital swabs</td>
<td>Transport Medium for Swabs (REF 956-CE, REF 987-CE) or Transport Medium with Mucolytic Agent (REF 952-CE, REF 953-CE)</td>
<td>DNA-sorb-AM</td>
<td>$1 \times 10^3$</td>
</tr>
</tbody>
</table>

13.2. Specificity
The analytical specificity of AmpliSens® *Treponema pallidum*-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 reactions were absent while testing human DNA samples and 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*, *Neisseria mucosa*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Toxoplasma gondii*, *HSV* types 1 and 2, *CMV*, and *HPV*.

$^3$ The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.
The clinical specificity of AmpliSens® *Treponema pallidum*-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 AmpliSens® *Treponema pallidum*-FRT PCR kit has been tested against predetermined specifications to ensure consistent product quality.
16. KEY TO SYMBOLS USED

- **REF** Catalogue number
- **LOT** Batch code
- **IVD** *In vitro* diagnostic medical device
- **VER** Version
- **Temperature limitation**
- **Manufacturer**
- **Date of manufacture**
- **Authorised representative in the European Community**
- **EC REP**
- **Caution**

- **Σ** Sufficient for
- **خوف من الشمس** Keep away from sunlight
- **NCA** Negative control of amplification
- **C−** Negative control of extraction
- **C+** Positive control of Amplification
- **IC** Internal control
List of Changes Made in the Instruction Manual

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