AmpliSens® *Trichomonas vaginalis*-FRT

PCR kit

Instruction Manual

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1. INTENDED USE
AmpliSens® *Trichomonas vaginalis*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Trichomonas vaginalis* DNA in the clinical material (urogenital swabs, urine samples, 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
*Trichomonas vaginalis* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers. In real-time PCR the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. 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® *Trichomonas vaginalis*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (IC)), which 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® *Trichomonas vaginalis*-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® *Trichomonas vaginalis*-FRT PCR kit is produced in 2 forms:

AmpliSens® *Trichomonas vaginalis*-FRT PCR kit variant FRT, [REF] R-B6(RG)-CE, [REF] R-B6(iQ)-CE.

AmpliSens® *Trichomonas vaginalis*-FRT PCR kit variant FRT-100 F, [REF] R-B6-F(RG,iQ)-CE.

AmpliSens® *Trichomonas vaginalis*-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 Trichomonas vaginalis (under wax)</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 directly to the sample/lysis mixture (see the DNA-sorb-AM REF K1-12-100-CE protocol).

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

AmpliSens® Trichomonas vaginalis-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 Trichomonas vaginalis</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>
<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 (IC) during the DNA extraction directly to the sample/lysis mixture (see the DNA-sorb-AM REF K1-12-100-CE protocol).

AmpliSens® Trichomonas vaginalis-FRT PCR kit variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
• Disposable powder-free gloves and laboratory coat.
• Pipettes (adjustable).
• Sterile pipette tips with aerosol filters up to 100 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), iCycler iQ or 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 PCR tubes with optical transparent domed caps if a plate-type instrument is used;
  b) 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® Trichomonas vaginalis-FRT** PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (urogenital swabs; urine (a sediment of the first portion of the morning specimen); prostate gland secretion).

7. WORKING CONDITIONS

**AmpliSens® Trichomonas vaginalis-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— (Negative control of Extraction).

⚠ 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. Prepare the required number of the tubes with PCR-mix-1-FL *Trichomonas vaginalis* 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 wax layer into each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-FL *Trichomonas vaginalis*.

**Variant FRT-100 F**

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 *Trichomonas vaginalis*, PCR-mix-2-FRT, and polymerase (TaqF), then centrifuge briefly.
   Take the required number of the tubes/stripes for amplification of DNA obtained 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 *Trichomonas vaginalis*;
   - 5.0*(N+1) µl of PCR-mix-2-FRT;
   - 0.5*(N+1) µl of polymerase (TaqF).
   Vortex the tube, then centrifuge briefly. Transfer 15 µl of the prepared mixture to each tube.

Steps 3 and 4 are required in both variants.

3. Add 10 µl of DNA samples obtained at the stage of DNA extraction.
4. Carry out 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−) reagent to the tube labeled C− (Negative Control of Extraction).

8.2.2. Amplification

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

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Cycles</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95</td>
<td>15 min</td>
<td>1</td>
<td>95</td>
<td>15 min</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>5 s</td>
<td>5</td>
<td>95</td>
<td>5 s</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20 s</td>
<td></td>
<td>60</td>
<td>20 s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>15 s</td>
<td></td>
<td>72</td>
<td>15 s</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>5 s</td>
<td>40</td>
<td>95</td>
<td>5 s</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20 s</td>
<td>Fluorescence acquiring</td>
<td>60</td>
<td>30 s</td>
<td>Fluorescence acquiring</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>15 s</td>
<td></td>
<td>72</td>
<td>15 s</td>
<td></td>
</tr>
</tbody>
</table>

Fluorescent signal is detected in the channels for 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 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 *Trichomonas vaginalis* DNA amplification product is detected in the channel for the FAM fluorophore;

– The signal of the Internal Control 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 $Ct$ value of the DNA sample in the corresponding column of the result grid.

Principle of interpretation is the following:

– *Trichomonas vaginalis* DNA is **detected** in a sample if the $Ct$ value is determined in the result grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

– *Trichomonas vaginalis* DNA is **not detected** in a sample if the $Ct$ value is not determined (absent) in the result grid in the channel for the FAM fluorophore (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, DT-96 or equivalent.
fluorescence curve does not cross the threshold line), whereas the $Ct$ value in the channel for JOE fluorophore is less than the specified boundary $Ct$ value.

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

⚠️ 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 channel for fluorophore</th>
</tr>
</thead>
<tbody>
<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

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

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

2. If the $Ct$ value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C−) in the channel for the FAM fluorophore, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which *Trichomonas vaginalis* DNA was detected.

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

### 11. TRANSPORTATION

**AmpliSens® Trichomonas vaginalis-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® *Trichomonas vaginalis*-FRT PCR kit (except for Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8°C when not in use. They are stable until the expiry date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

- Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C when not in use.
- PCR-mix-1-FL *Trichomonas vaginalis* should be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

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

<table>
<thead>
<tr>
<th>Clinical material</th>
<th>Transport medium</th>
<th>Nucleic acid extraction kit</th>
<th>PCR kit</th>
<th>Analytical sensitivity, GE/ml</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 (REF 952-CE, REF 953-CE)</td>
<td>DNA-sorb-AM</td>
<td>PCR kit variant FRT</td>
<td>5 x 10²</td>
</tr>
<tr>
<td>Urine (pretreatment is required)</td>
<td>–</td>
<td>DNA-sorb-AM</td>
<td>PCR kit variant FRT</td>
<td>1 x 10³</td>
</tr>
</tbody>
</table>

13.2. Specificity

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

Nonspecific reactions were absent while testing human DNA samples 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*, *Neisseria flava*, *Neisseria subflava*, *Neisseria sicca*, *Neisseria mucosa*, *Neisseria gonorrhoeae*, *Chlamydia thrachomatis*, *Treponema pallidum*, *Toxoplasma gondii*, *HSV* types 1 and 2, *CMV*, and *HPV*.

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³ Genome equivalents (GE) of the pathogen agent per 1 ml of a sample placed in the transport medium
The clinical specificity of AmpliSens® *Trichomonas vaginalis*-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” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

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

**REF**
Catalogue number

**Caution**

**LOT**
Batch code

**Sufficient for**

**IVD**
*In vitro* diagnostic medical device

**Expiration Date**

**VER**
Version

**Consult instructions for use**

**Temperature limitation**

**Keep away from sunlight**

**Manufacturer**

**NCA**
Negative control of amplification

**Date of manufacture**

**C–**
Negative control of extraction

**Authorised representative in the European Community**

**C+**
Positive control of Amplification

**IC**
Internal control
## List of Changes Made in the Instruction Manual

<table>
<thead>
<tr>
<th>VER</th>
<th>Location of changes</th>
<th>Essence of changes</th>
</tr>
</thead>
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<td>29.06.11 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>
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<td>Corrections in accordance with the template</td>
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<td>9. Data analysis</td>
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<td>10. Troubleshooting</td>
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