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1. INTENDED USE
AmpliSens® *T. vaginalis / N. gonorrhoeae*-MULTIPRIME-FRT PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of *Trichomonas vaginalis* and *Neisseria gonorrhoeae* DNA in the clinical material (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; urine samples, 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
*T. vaginalis / N. gonorrhoeae* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *T. vaginalis / N. gonorrhoeae* 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® *T. vaginalis / N. gonorrhoeae*-MULTIPRIME-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® *T. vaginalis / N. gonorrhoeae*-MULTIPRIME-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® *T. vaginalis / N. gonorrhoeae*-MULTIPRIME-FRT PCR kit is produced in 2 forms:

- **AmpliSens® *T. vaginalis / N. gonorrhoeae*-MULTIPRIME-FRT** PCR kit variant FRT [REF] R-B65(RG)-CE.
- **AmpliSens® *T. vaginalis / N. gonorrhoeae*-MULTIPRIME-FRT** PCR kit variant FRT-100 F [REF] R-B65-F(RG,iQ)-CE / [VER] 29.12.12–15.03.18 / Page 3 of 13
**AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-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 T.vaginalis / N.gonorrhoeae (ready-to-use single-dose test tubes (under wax))</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>colorless 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-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE protocol).

**AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-FRT** PCR kit is intended for 110 reactions (including controls).

**AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-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 T.vaginalis / N.gonorrhoeae</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 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-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE protocol).

**AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-FRT** PCR kit 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 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).
- Disposable polypropylene tubes:
  a) thin-walled 0.2-ml PCR tubes with 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® T.vaginalis / N.gonorrhoeae-MULTIPRIME-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; urine samples (a sediment of the first portion of the morning specimen); prostate gland secretion).

7. WORKING CONDITIONS
AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-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:

- 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

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.

8.2.1. Preparing tubes for PCR

**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 *T. vaginalis / N.gonorrhoeae* 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 *T. vaginalis / N.gonorrhoeae*.

**Variant FRT-100 F**

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 *T. vaginalis / N.gonorrhoeae*, PCR-mix-2-FRT, and polymerase (TaqF) and then centrifuge briefly.

   Take the required number of tubes/stripes for amplification of the DNA obtained 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 *T. vaginalis / N.gonorrhoeae*,
   - 5.0-(N+1) µl of PCR-mix-2-FRT,
   - 0.5-(N+1) µl of polymerase (TaqF).

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

Steps 3 and 4 are required in both variants.

3. Using tips with aerosol filter, add 10 µl of DNA samples obtained at the DNA extraction stage.

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** 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></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>95</td>
<td>15 min</td>
<td>1</td>
<td>95</td>
<td>15 min</td>
<td>1</td>
</tr>
<tr>
<td>Cycling</td>
<td>60</td>
<td>20 s</td>
<td>5</td>
<td>60</td>
<td>20 s</td>
<td>5</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>Cycling 2</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 Fluorescence acquiring</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>
</tbody>
</table>

Fluorescent signal is detected in the channels designed for the FAM, JOE and ROX 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 three channels:

– The signal of the *Trichomonas vaginalis* DNA amplification product is detected in the channel for the FAM fluorophore.

– The signal of the *Neisseria gonorrhoeae* DNA amplification product is detected in the channel for the JOE fluorophore.

– The signal of the IC DNA amplification product is detected in the channel for the ROX fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the

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1 For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q.
2 For example, iQ5, Mx3000P, Mx3000.
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:

- **Trichomonas vaginalis** DNA is detected 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 typical exponential growth of fluorescence.

- **Neisseria gonorrhoeae** DNA is detected if the Ct value is determined in the results grid in the channel for the JOE fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

- **Trichomonas vaginalis** and **Neisseria gonorrhoeae** DNA are not detected in a sample if the Ct value is not determined (absent) in the channels for FAM and JOE fluorophores, whereas the Ct value determined in the channel for the ROX 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 channels for FAM, JOE or ROX fluorophores. In such cases, the PCR analysis should be repeated.

⚠️ 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FAM, JOE</td>
</tr>
<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 channels for the FAM and/or JOE and/or ROX fluorophores is greater than the boundary Ct value or absent, the amplification should be repeated for all samples in which the boundary Ct is absent in the channels for the FAM and/or JOE and/or ROX fluorophores.
2. If the Ct value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channels for the FAM and/or JOE fluorophores, the PCR analysis should be repeated for all samples in which the Ct value is determined in the channels for the FAM and/or JOE fluorophores.

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

11. TRANSPORTATION

AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-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® T.vaginalis / N.gonorrhoeae-MULTIPRIME-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® T.vaginalis / N.gonorrhoeae-MULTIPRIME-FRT PCR kit are stable until the expiry date 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 when not in use.
- PCR-mix-1-FL T.vaginalis / N.gonorrhoeae is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-FRT PCR kit is the following:

<table>
<thead>
<tr>
<th>Clinical material</th>
<th>Transport medium</th>
<th>Nucleic acid extraction kit</th>
<th>Microorganism</th>
<th>Sensitivity, GE/ml³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neisseria gonorrhoeae</td>
<td>5x10²</td>
</tr>
<tr>
<td>Urine⁴</td>
<td></td>
<td>DNA-sorb-AM</td>
<td>Trichomonas vaginalis</td>
<td>10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neisseria gonorrhoeae</td>
<td>10³</td>
</tr>
</tbody>
</table>

The analytical sensitivity of each microorganism does not change even at high concentrations of the other microorganism (to 10³ GE/ml).

³ The quantity of genome equivalents of microorganism per 1 ml of the sample.
⁴ Treatment is needed.
13.1. Specificity

The analytical specificity of AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-FRT PCR kit is ensured by the selection of specific primers and probes as well as strict 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 while testing human DNA samples as well as a DNA panel of the following microorganisms: Gardnerella vaginalis, Lactobacillus spp., Escherichia coli, Staphylococcus spp., Streptococcus spp., Candida albicans, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Chlamydia trachomatis, Neisseria spp., Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV types 1 and 2, CMV, and HPV.

The clinical specificity of AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-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 compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of AmpliSens® T.vaginalis / N.gonorrhoeae-MULTIPRIME-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>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>IVD</td>
<td><em>In vitro</em> diagnostic medical device</td>
</tr>
<tr>
<td>VER</td>
<td>Version</td>
</tr>
<tr>
<td></td>
<td>Temperature limitation</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td></td>
<td>Date of manufacture</td>
</tr>
<tr>
<td>EC</td>
<td>Authorized representative in the European Community</td>
</tr>
<tr>
<td></td>
<td>Authorized representative in the European Community</td>
</tr>
</tbody>
</table>

- **Caution**: Sufficient for
- **Expiration Date**: Keep away from sunlight
- **Consult instructions for use**: Negative control of amplification
- **Negative control of extraction**: Positive control of Amplification
- **Internal control**:
<table>
<thead>
<tr>
<th>VER</th>
<th>Location of changes</th>
<th>Essence of changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.06.11 LA</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>
</tr>
<tr>
<td>17.02.16 PM</td>
<td>Through the text</td>
<td>Corrections in accordance with the template</td>
</tr>
<tr>
<td></td>
<td>8.1. DNA extraction</td>
<td>Information about controls of extraction was added</td>
</tr>
<tr>
<td></td>
<td>9. Data analysis</td>
<td>The sections were rewritten</td>
</tr>
<tr>
<td></td>
<td>10. Troubleshooting</td>
<td></td>
</tr>
<tr>
<td>15.03.18 PM</td>
<td>Footer, 3. Content</td>
<td>REF R-B65(iQ)-CE was deleted</td>
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