

AmpliSens® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-MULTIPRIME-FRT PCR kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

| | | | |
|--|---|------------|-----------------------------------|
| | Catalogue number | | Caution |
| | Batch code | | Sufficient for |
| | <i>In vitro</i> diagnostic medical device | | Use-by Date |
| | Version | | Consult instructions for use |
| | Temperature limit | | Keep away from sunlight |
| | Manufacturer | NCA | Negative control of amplification |
| | Date of manufacture | C- | Negative control of extraction |
| | Authorized representative in the European Community | C+ | Positive control of amplification |
| | | IC | Internal control |

1. INTENDED USE

AmpliSens® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-MULTIPRIME-FRT PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of the DNA of *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis* in the clinical material (urogenital, rectal, and oropharyngeal swabs; eye conjunctival discharge; prostatic gland secretion; and urine samples) by using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

T.vaginalis, *N.gonorrhoeae*, and *C.trachomatis* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific regions using specific *T.vaginalis*, *N.gonorrhoeae*, and *C.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® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-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* / *C.trachomatis*-MULTIPRIME-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "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.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR. The results of amplification are registered in the following fluorescence channels.

Table 1

| Channel for fluorophore | FAM | JOE | ROX | Cy5 |
|-------------------------|------------------------------------|----------------------|----------------------|-------------------------------------|
| DNA-target | <i>T.vaginalis</i> | <i>N.gonorrhoeae</i> | <i>C.trachomatis</i> | Internal Control-FL |
| Target gene | DNA repeats for PCR identification | 16s rRNA gene | cryptic plasmid | genetically engineered construction |

3. CONTENT

AmpliSens® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-MULTIPRIME-FRT PCR kit is produced in 1 form:

variant FRT-100 F R-B83-F(RG,iQ)-CE.

Variant FRT-100 F includes:

| Reagent | Description | Volume, ml | Quantity |
|---|---|------------|----------|
| PCR-mix-1-FL <i>T.vaginalis</i> / <i>N.gonorrhoeae</i> / <i>C.trachomatis</i> | clear liquid from colorless to light lilac colour | 1.2 | 1 tube |
| PCR-mix-2-FRT | colorless clear liquid | 0.3 | 2 tubes |
| Polymerase (TaqF) | colorless clear liquid | 0.03 | 2 tubes |
| Positive Control complex (C+) | colorless clear liquid | 0.2 | 1 tube |
| DNA-buffer | colorless clear liquid | 0.5 | 1 tube |
| Negative Control (C-)* | colorless clear liquid | 1.2 | 1 tube |
| Internal Control-FL (IC)** | colorless clear liquid | 1.0 | 1 tube |

* 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 K1-12-100-CE protocol).

Variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- 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 iQ or iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
 - a) 0.2-ml PCR tubes with optical transparent domed or flat 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 to 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- 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

NOTE: 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* / *C.trachomatis*-MULTIPRIME-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (urogenital swabs, rectal swabs, oropharyngeal swabs, eye conjunctival discharge, prostate gland secretion, urine (use the first portion pellet of morning urine)).

7. WORKING CONDITIONS

AmpliSens® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-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)**.

NOTE: Extract 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-100 F

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

1. Thaw the required number of tubes with **PCR-mix-2-FRT**. Vortex the tubes with **PCR-mix-1-FL *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis***, **PCR-mix-2-FRT**, and **polymerase (TaqF)** and then centrifuge briefly.

Take the required number of tubes/strips for amplification of the cDNA 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* / *C.trachomatis***,
 - 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 carried out 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 2

| AmpliSens-1 amplification program | | | | | | |
|-----------------------------------|-------------------------------------|------------------------|--------|-------------------------------------|------------------------|--------|
| Step | Rotor-type instruments ¹ | | | Plate-type instruments ² | | |
| | Temperature, °C | Time | Cycles | Temperature, °C | Time | Cycles |
| 1 | 95 | 15 min | 1 | 95 | 15 min | 1 |
| 2 | 95 | 5 s | 5 | 95 | 5 s | 5 |
| | 60 | 20 s | | 60 | 20 s | |
| | 72 | 15 s | | 72 | 15 s | |
| 3 | 95 | 5 s | 40 | 95 | 5 s | 40 |
| | 60 | 20 s | | 60 | 30 s | |
| | | Fluorescence acquiring | | | Fluorescence acquiring | |
| | 72 | 15 s | | 72 | 15 s | |

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, and Cy5 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* 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 four 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 *Chlamydia trachomatis* DNA amplification product is detected in the channel for the ROX fluorophore.
- The signal of the IC DNA amplification product is detected in the channel for the Cy5 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:

- *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.
- *Chlamydia trachomatis* DNA is **detected** if the Ct value is determined in the results grid in the channel for the ROX fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* DNA is **not detected** in a sample if the Ct value is not determined (absent) in the channels for FAM, JOE and ROX fluorophores (the fluorescence curve does not cross the threshold line), whereas the Ct value determined in the channel for the Cy5 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 Cy5 fluorophore and FAM, JOE, ROX fluorophores. In such cases, the PCR analysis should be repeated for corresponding sample.

NOTE: 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 Positive and Negative Controls of Amplification as well as for the Negative Control of Extraction are correct (see Table 3).

Table 3

| Results for controls | | | |
|----------------------|-------------------|---|-----------------|
| Control | Stage for control | Ct value in the channel for fluorophore | |
| | | FAM, JOE, ROX | Cy5 |
| C– | DNA extraction | Absent | <boundary value |
| NCA | PCR | Absent | Absent |
| C+ | PCR | <boundary value | <boundary value |

10. TROUBLESHOOTING

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

1. If the Ct value for the Positive Control of Amplification (C+) in the channels for the FAM and/or JOE and/or ROX fluorophores is absent or greater than the boundary Ct value, the amplification should be repeated for all samples in which Ct value is absent in the respective channel.
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 and/or ROX fluorophores, the PCR analysis starting from the DNA extraction stage should be repeated for all samples in which Ct value is determined in the respective channel.

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* / *C.trachomatis*-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* / *C.trachomatis*-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* / *C.trachomatis*-MULTIPRIME-FRT PCR kit** are stable until the expiration date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

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

NOTE: PCR-mix-1-FL *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis* is to be kept away from light.

¹ For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.

² For example, iCycler iQ5, Mx3000P, Mx3000, or equivalent.

13. SPECIFICATIONS

13.1. Sensitivity

| Clinical material | Nucleic acid extraction kit | PCR kit | Pathogen agent | Sensitivity, GE/ml ³ |
|-------------------------------|-----------------------------|-------------------|------------------------------|---------------------------------|
| Urogenital swabs ⁴ | DNA-sorb-AM | variant FRT-100 F | <i>Trichomonas vaginalis</i> | 5x10 ² |
| | | | <i>Neisseria gonorrhoeae</i> | 5x10 ² |
| | | | <i>Chlamydia trachomatis</i> | 5x10 ² |
| Urine ⁵ | DNA-sorb-AM | variant FRT-100 F | <i>Trichomonas vaginalis</i> | 1x10 ³ |
| | | | <i>Neisseria gonorrhoeae</i> | 1x10 ³ |
| | | | <i>Chlamydia trachomatis</i> | 1x10 ³ |

Analytical sensitivity of the PCR kit in the case of each microorganism does not change even at high concentrations of two other microorganisms (to 10⁹ GE/ml).

13.2. Specificity

The analytical specificity of **AmpliSens® T.vaginalis / N.gonorrhoeae / C.trachomatis - MULTIPRIME-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 in tests of human DNA samples and DNA samples 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 type 1 and 2, CMV, HPV.

The clinical specificity of **AmpliSens® T.vaginalis / N.gonorrhoeae / C.trachomatis - MULTIPRIME-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

- Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", 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.
- Guidelines "Real-Time PCR Detection of STIs and Other Reproductive Tract Infections", issued 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 / C.trachomatis-MULTIPRIME-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

| VER | Location of changes | Essence of changes |
|----------------|-------------------------------|---|
| 24.09.15 ME | Through the text | Corrections in accordance with the template |
| | 1. Intended use | The clinical material was specified |
| | 6. Sampling and handling | |
| | 3. Content, Footer | REF R-B83(iQ)-CE was deleted |
| 25.12.17 ME | 3. Content | The color of the reagent was specified |
| 05.12.18 EM | 2. Principle of PCR detection | The table with targets and the information about the enzyme UDG were added |
| | Through the text | The text formatting was changed |
| 27.02.20 PM | Footer | The phrase "Not for use in the Russian Federation" was added |
| 30.10.20 MA | Footer, 3. Content | The information about variant FRT REF R-B83(RG)-CE was deleted |
| 01.03.21 EM | — | The name, address and contact information for Authorized representative in the European Community was changed |

AmpliSens®



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³ The quantity of genome equivalents of microorganism per 1 ml of the sample placed in the transport medium specified.

⁴ Urogenital swabs are to be placed into **Transport Medium for Swabs** (**REF** 956-CE, **REF** 987-CE) or **Transport Medium with Mucolytic Agent** (**REF** 952-CE).

⁵ Pretreatment is required.