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For Professional Use Only

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

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

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

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

2. PRINCIPLE OF PCR DETECTION

Detection of *T.vaginalis, N.gonorrhoeae,* and *C.trachomatis* by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific regions using specific *T.vaginalis, N.gonorrhoeae,* and *C.trachomatis* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. The real-time monitoring of the 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-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 separation of nucleotides and Taq-polymerase by using a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *T.vaginalis / N.gonorrhoeae / C.trachomatis*-MULTIPRIME-FRT PCR kit is produced in 3 forms:

AmpliSens® *T.vaginalis / N.gonorrhoeae / C.trachomatis*-MULTIPRIME-FRT PCR kit variant FRT for use with RG, **REF** R-B83(RG)-CE;

AmpliSens® *T.vaginalis / N.gonorrhoeae / C.trachomatis*-MULTIPRIME-FRT PCR kit variant FRT for use with iQ, **REF** R-B83(iQ)-CE;

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

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

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

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *T.vaginalis / N.gonorrhoeae / C.trachomatis* (ready-to-use single-dose test tubes (*under wax)*)	colorless clear liquid	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
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 **REF** K1-12-100-CE protocol).

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

AmpliSens® *T.vaginalis / N.gonorrhoeae / C.trachomatis*-MULTIPRIME-FRT PCR kit variant FRT-100 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *T.vaginalis / N.gonorrhoeae / C.trachomatis*	colorless clear liquid	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 **REF** K1-12-100-CE protocol).

AmpliSens® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-MULTIPRIME-FRT PCR kit, variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia), iCycler iQ or iQ5 (Bio-Rad, USA), or equivalent).
- Disposable polypropylene tubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste 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 new tips for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.

- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water and seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area in which 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 is described in the manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens® ***T.vaginalis / N.gonorrhoeae / C.trachomatis*****-MULTIPRIME-FRT** PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from:

- urogenital swabs;
- rectal swabs;
- pharyngeal swabs;
- eye conjunctival discharge;
- prostate gland secretion;
- urine (use the first portion of the morning specimen).

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.

- Other nucleic acid extraction kits, recommended by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being [2].

Extract DNA according to the manufacturer's instructions.

8.2. Preparing the PCR

Variant FRT

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

8.2.1. Preparing tubes for PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FL *T.vaginalis / N.gonorrhoeae / C.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 of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *T.vaginalis / N.gonorrhoeae / C.trachomatis***.

Variant FRT-100 F

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

1. Vortex tubes with **PCR-mix-1-FL *T.vaginalis / N.gonorrhoeae / C.trachomatis,* PCR-mix-2-FRT**, and **polymerase (TaqF)** then centrifuge shortly.
2. Prepare the required number of the tubes for amplification of DNA from clinical and control samples (0.2-ml tubes for a 36-well rotor or 0.1-ml strips for a 72-well rotor).

Unfreeze PCR-mix-2-FRT before mixing.

3. For carrying out N reactions (including 2 controls), mix in a new tube: **10*(N+1) µl of PCR-mix-1-FL *T.vaginalis / N.gonorrhoeaea / C.trachomatis*, 5.0*(N+1) µl of PCR-mix-2-FRT** and **0.5*(N+1) µl of polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer **15 µl** of the prepared mixture to each tube.

Steps 4 and 5 are carried out in both variants.

4. Using tips with aerosol barrier, add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes.
5. 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 Negative Control of Extraction (C–)**

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

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

Table 1

AmpliSens-1 amplification program

Step	**Rotor-type instruments[1]**			**Plate-type instruments[2]**		
	Temperature, °C	***Time***	***Cycle repeats***	***Temperature, °C***	***Time***	***Cycle repeats***
Hold	95	15 min	1	95	15 min	1
Cycling	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
Cycling 2	95	5 s	40	95	5 s	40
	60	**20 s (fluorescence detection)**		60	**30 s (fluorescence detection)**	
	72	15 s		72	15 s	

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM, JOE, ROX, and Cy5 fluorescence channels.

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

- ***Trichomonas vaginalis* DNA** amplification product is detected is in the **FAM** fluorescence channel,
- ***Neisseria gonorrhoeae* DNA** amplification product is detected in the **JOE** fluorescence channel,
- ***Chlamydia trachomatis* DNA** is detected in the **ROX** channel,
- **Internal Control DNA** is detected in the **Cy5** channel.

Interpretation of results

The results are interpreted by the software of the PCR instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

The result of the analysis is considered reliable only if the results obtained for Positive and

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[2] For example, iCycler iQ5, Mx3000P, Mx3000, or equivalent.

Negative Controls of Amplification as well as for the Negative Control of Extraction are correct.

Table 2

Results for controls

Control	Stage for control	Ct in channels		Interpretation
		FAM, JOE, ROX	Cy5	
C–	DNA extraction	Neg	Pos (< boundary Ct value)*	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary Ct value)*	Pos (< boundary Ct value)*	OK

* For boundary Ct values for samples, Negative Control of Extraction, and Positive Control of Amplification, see the **Important Product Information Bulletin**.

1. The sample is considered to be **positive** for ***Trichomonas vaginalis*** if its Ct value is detected in the results grid in the FAM channel. Moreover, the fluorescence curve should cross the threshold line in the region of exponential fluorescence growth.
2. The sample is considered to be **positive** for ***Neisseria gonorrhoeae*** if its Ct value is detected in the results grid in the JOE channel. Moreover, the fluorescence curve should cross the threshold line in the region of exponential fluorescence growth.
3. The sample is considered to be **positive** for ***Chlamydia trachomatis*** if its Ct value is detected in the results grid in the ROX channel. Moreover, the fluorescence curve should cross the threshold line in the region of exponential fluorescence growth.
4. The sample is considered to be **negative** for *Trichomonas vaginalis, Neisseria gonorrhoeae*, and *Chlamydia trachomatis* if its Ct value is not detected in the results grid (the fluorescence curve does not cross the threshold line) in FAM, JOE, and ROX channels and the Ct value does not exceed the boundary Ct value in the results grid in the Cy5 channel.

10. TROUBLESHOOTING

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

- If no signal is detected for the sample in the channels intended for pathogen detection (FAM, JOE, and ROX) and in the channel for IC detection (Cy5), the result of analysis is **invalid**. PCR should be repeated.
- If no signal is detected for the Positive Control of Amplification (C+) or its Ct value exceeds the boundary Ct value in FAM, JOE and ROX channels, PCR reaction should

be repeated for the samples without detected signal in the channels.

- If the positive signal in negative controls (C– or NCA) in the channels for detection of pathogen DNA is detected, analysis must be repeated for the samples in which a Ct value was detected.

11. TRANSPORTATION

AmpliSens® *T.vaginalis / N.gonorrhoeae / C.trachomatis*-MULTIPRIME-FRT PCR kit should be transported at 2–8 ºC 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 (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at the temperature 2–8 °C when not in use. All components of the **AmpliSens® *T.vaginalis / N.gonorrhoeae / C.trachomatis*-MULTIPRIME-FRT** PCR kit are stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

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

Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Nucleic acid extraction kit	PCR kit	Microorganism	Sensitivity, GE/ml[3]
Urogenital swabs[4]	DNA-sorb-AM	PCR kit variants FRT and FRT-100 F	*Trichomonas vaginalis*	$5x10^2$
			Neisseria gonorrhoeae	$5x10^2$
			Chlamydia trachomatis	$5x10^2$
Urine[5]	DNA-sorb-AM	PCR kit variants FRT and FRT-100 F	*Trichomonas vaginalis*	$1x10^3$
			Neisseria gonorrhoeae	$1x10^3$
			Chlamydia trachomatis	$1x10^3$

[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.

Analytical sensitivity of the PCR kit in the case of each microorganism does not change even at high concentrations of two other microorganisms (to 10^9 GE/ml).

13.2. Specificity

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

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, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Mycoplasma genitalium, Treponema pallidum, Toxoplasma gondii, HSV* type 1 and 2, *CMV,* and *HPV*.

14. REFERENCES

1. 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, Moscow, 2008.
2. 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.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
RUO	Research Use Only		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
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
08.07.13 FN	Cover page	IVD symbol was changed to RUO symbol
	Key to Symbols Used	
	Text	“Federal Budget Institution of Science” was changed to “Federal Budget Institute of Science”