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

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
RUO	Research Use Only		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C–	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
	Caution	IC	Internal control

1. INTENDED USE

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

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

N.gonorrhoeae / *C.trachomatis* / *M.genitalium* / *T.vaginalis* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific regions using specific *N.gonorrhoeae* / *C.trachomatis* / *M.genitalium* / *T.vaginalis* 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® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-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® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-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	Cy5.5
DNA-target	*N.gonorrhoeae*	*C.trachomatis*	*M.genitalium*	Internal Control-FL	*T.vaginalis*
Target gene	*16s rRNA* gene	*cryptic plasmid*	*gyrB* gene	genetically engineered construction	DNA repeats for PCR identification

3. CONTENT

AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-MULTIPRIME-FRT PCR kit is produced in 2 forms:

variant FRT-100 F REF R-B61-F(RG)-CE;

variant FRT-100 F in bulk[1] REF R-B61-F(RG)-CE-B.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*	clear liquid from colorless to blue grey colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	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** protocol).

Variant FRT-100 F 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 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany).
- Disposable polypropylene 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes.
- 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 barriers 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.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-MULTIPRIME-FRT PCR kit is intended for analysis of DNA extracted with DNA extraction kits from the biological material (urogenital swabs, rectal swabs, oropharyngeal swabs, conjunctival discharge, prostate gland secretion, urine samples).

7. WORKING CONDITIONS

AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-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**.
- For other nucleic acid extraction kits see Guidelines [2].

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

1. Prepare the required number of tubes or strips for amplification of DNA from test and control samples.
2. For carrying out N reactions (including 2 controls), mix in a new tube: **10·(N+1) µl** of **PCR-mix-1-FL *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*, 5.0·(N+1) µl** of **PCR-mix-2-FRT** and **0.5·(N+1) µl** of **polymerase (TaqF)**. Vortex the tube, then centrifuge briefly. Transfer **15 µl** of the prepared mixture into each tube.
3. Add **10 µl** of **DNA** samples obtained at the DNA extraction stage into 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 **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	Temperature, °C	Time	Cycles
Hold	**95**	**15 min**	**1**
Cycling	**95**	**5 s**	**5**
	60	**20 s**	
	72	**15 s**	
Cycling 2	**95**	**5 s**	**40**
	60	**20 s (fluorescence detection)**	
	72	**15 s**	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, Cy5 and Cy5.5 fluorophores.

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 five channels:

– The signal of the ***Neisseria gonorrhoeae*** DNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the ***Chlamydia trachomatis*** DNA amplification product is detected in the channel for the JOE fluorophore.
– The signal of the ***Mycoplasma genitalium*** 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.
– The signal of the ***Trichomonas vaginalis*** DNA amplification product is detected in the channel for the Cy5.5 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:

– ***Neisseria gonorrhoeae*** 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.
– ***Chlamydia trachomatis*** 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.
– ***Mycoplasma genitalium*** 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*** DNA is **detected** if the *Ct* value is determined in the results grid in the channel for the Cy5.5 fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– ***Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium*** and ***Trichomonas vaginalis*** DNA is **not detected** in a sample if the *Ct* values are not determined (absent) in the channels for the FAM, JOE, ROX and Cy5.5 fluorophores, 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 in the channel for the Cy5 fluorophore is not determined (absent) or greater than the specified boundary *Ct* value, and the *Ct* value in the channels for the FAM, JOE, ROX and Cy5.5 fluorophores is not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR should be repeated. If the same result is obtained in the second run, the analysis should be repeated starting from the DNA extraction stage.

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, Cy5.5	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 no signal is detected for Positive Control of Amplification (C+) or the signal is greater than the specified boundary *Ct* value in the channels for the FAM, ROX, JOE and Cy5.5 fluorophores, PCR should be repeated for all samples for which *Ct* values in these channels were not detected.
2. If a *Ct* value is determined for the Negative Control of Extraction (C–) and/or for the Negative Control of Amplification (NCA) in the channels for the FAM, ROX, JOE and Cy5.5 fluorophores, PCR analysis should be repeated for all samples for which a *Ct* value in these channels was determined.
3. If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample with a fluorescence curve without the area of typical exponential growth (the fluorescence curve is approximately linear), this may indicate incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. If such result was obtained in the presence of the correct setting of threshold line, PCR analysis of the sample should be repeated.

11. TRANSPORTATION

AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-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® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-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® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-MULTIPRIME-FRT** PCR kit are stable until the expiry date stated on the label. PCR kit **variant FRT-100 F** can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit **variant FRT-100 F** should be unpacked in accordance with the storage temperatures for each component. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

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

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

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Nucleic acid extraction kit	PCR kit	Microorganism	Sensitivity, GE/ml[2]
Cervical or urethral swabs[3]	**DNA-sorb-AM**	variant FRT-100 F	*Neisseria gonorrhoeae*	$5x10^2$
			Chlamydia trachomatis	$5x10^2$
			Mycoplasma genitalium	$1x10^3$
			Trichomonas vaginalis	$5x10^2$
Urine[4]	**DNA-sorb-AM**	variant FRT-100 F	*Neisseria gonorrhoeae*	$1x10^3$
			Chlamydia trachomatis	$1x10^3$
			Mycoplasma genitalium	$2x10^3$
			Trichomonas vaginalis	$1x10^3$

NOTE: The analytical sensitivity of each microorganism does not change even in the case of high concentration of the other analyzed microorganisms – up to 10^9 GE/ml.

13.2. Specificity

The analytical specificity of **AmpliSens® *N. gonorrhoeae / C. trachomatis / M. genitalium / T. vaginalis*-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 while testing 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* 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.
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.

[2] The quantity of genome equivalents of microorganism per 1 ml of the sample placed in a transport medium specified.
[3] Cervical and urethral swabs are to be placed in the **Transport Medium for Swabs** or **Transport Medium with Mucolytic Agent**.
[4] Pretreatment is required for urine samples.

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 the **AmpliSens® *N.gonorrhoeae / C.trachomatis/ M.genitalium / T.vaginalis*-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
06.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
	Catalogue number	R-B61(RG)-CE, R-B61-F(RG)-CE were deleted
	CONTENT	Forms AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-multiprime-FRT, **REF** R-B61(RG)-CE, AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-multiprime-FRT PCR kit variant FRT-100 F, **REF** R-B61-F(RG)-CE were deleted
	CONTENT	Information about variant FRT (with aliquoted reagents) was deleted
	Preparing PCR	
	Key to Symbols Used	The explanation of symbols was corrected
21.06.11 VV	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science "Central Research Institute for Epidemiology"
25.12.12 LA	Cover page	IVD symbol was replaced by RUO symbol
	16. Key to symbols used	
27.07.16 PM	Text	Corrections according to the template
	1. Intended use	The clinical material was specified
	6. Sampling and handling	
	3. Content	The volume of PCR-mix-1-FL *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis* for PCR kit variant FRT-100 F was changed from 1.1 to 1.2 ml
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
	13. Specifications	The table with analytical sensitivity was added
		The information of absence of nonspecific responses was added
25.12.17 PM	3. Content	The color of the reagent was specified
03.06.21 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
	Footer	The phrase "For research use only. Not for diagnostic procedures" was added
22.06.23 EM	3. Content Footer	REF R-B61-F(RG)-CE was added
20.03.25 HM	2. Principle of PCR detection	Information in Table 1 was corrected (IC is detected in the channel for the Cy5 fluorophore, *T.vaginalis* - in the channel for the C5.5 fluorophore)

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