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IVD

For Professional Use Only

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE 3
2. PRINCIPLE OF PCR DETECTION 3
3. CONTENT 3
4. ADDITIONAL REQUIREMENTS 5
5. GENERAL PRECAUTIONS 5
6. SAMPLING AND HANDLING 6
7. WORKING CONDITIONS 6
8. PROTOCOL 6
9. DATA ANALYSIS 8
10. TROUBLESHOOTING 9
11. TRANSPORTATION 9
12. STABILITY AND STORAGE 9
13. SPECIFICATIONS 10
14. REFERENCES 11
15. QUALITY CONTROL 11
16. KEY TO SYMBOLS USED 12

1. INTENDED USE

AmpliSens® ***C.trachomatis / Ureaplasma / M.genitalium*****-MULTIPRIME-FRT** PCR kit is an *in vitro* nucleic acid amplification test for multiplex detection of *Chlamydia trachomatis, Ureaplasma* (*U.parvum* and *U.urealyticum*) and *Mycoplasma genitalium* DNA in clinical materials (urogenital, rectal and pharyngeal swabs; 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

C.trachomatis / *Ureaplasma* / *M.genitalium* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *C.trachomatis* / *Ureaplasma* / *M.genitalium* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which 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®** ***C.trachomatis / Ureaplasma / M.genitalium*****-MULTIPRIME-FRT** PCR kit is a qualitative test that contains the Internal Control (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®** ***C.trachomatis / Ureaplasma / M.genitalium*****-MULTIPRIME-FRT** PCR kit uses “hot-start”, which greatly reduces 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 ºC. Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

3. CONTENT

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

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FRT PCR kit variant FRT, **REF** R-B46-(iQ)-CE and **REF** R-B46-(RG)-CE.

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FRT PCR kit variant FRT-100 F, **REF** R-B46-F(RG,iQ)-CE.

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT PCR kit, variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium* 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® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT PCR kit variant FRT is intended for 110 reactions, including controls.

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT PCR kit, variant FRT-100 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium*	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® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-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 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 iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA); DT-96 (DNA-technology, Russia), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2- or 0.1-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 tip 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 other suitable disinfectant.
- Avoid contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes 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 techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. 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 manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FRT PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from urogenital, rectal and pharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples.

7. WORKING CONDITIONS

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-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 (see **Guidelines**).

Extract DNA according to the manufacturer's instructions.

8.2. Preparing 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 *C.trachomatis / Ureaplasma / M.genitalium*** 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 *C.trachomatis / Ureaplasma / M.genitalium***.

Variant FRT-100 F

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

1. 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).
2. For carrying out N reactions (including 2 controls), mix in a new tube: **10*(N+1) µl of PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium*, 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 mix to each tube.

Unfreeze PCR-mix-2-FRT before mixing.

Steps 3 and 4 apply to both variants.

3. Using tips with aerosol barrier add **10 µl** of **DNA** obtained from clinical or control samples 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** to the tube labeled C+ (Positive Control of Amplification).

8.2.2. Amplification

Program the thermocycler according to **Manufacturer's manual**, **Guidelines,** and Table 1.

Table 1

AmpliSens-1 RG amplification program

Step	Rotor type instruments[1]			Plate type instruments[2]		
	Temperature, °C	*Time*	*Cycles*	*Temperature, °C*	*Time*	*Cycles*
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 (fluore-scence detection)**		60	**30 s (fluore-scence detection)**	
	72	15 s		72	15 s	

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

9. DATA ANALYSIS

Chlamydia trachomatis **DNA** amplification product is detected in the **FAM/Green** fluorescence channel, ***Ureaplasma*** **spp.** *(**U.parvum*** and ***U.urealyticum**)* **DNA** is detected in the **JOE/Yellow/HEX** channel, ***Mycoplasma genitalium*** **DNA** is detected in the **ROX/Orange** channel, and the Internal Control is detected in the **Cy5/Red** channel.

9.1. Results interpretation

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

The results of the analysis are considered reliable only if the results obtained for both Positive and 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 channel FAM/Green, JOE/Yellow/HEX, ROX/Orange	Ct channel Cy5/Red	Interpretation
C–	DNA extraction	Neg	Pos (< boundary value) *	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value) *	Pos (< boundary value) *	OK

1. The sample is considered to be positive for *Chlamydia trachomatis* if its Ct value is defined in the results grid (the fluorescence curve crosses the threshold line) in the FAM/Green channel.

[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.

2. The sample is considered to be positive for *Ureaplasma* spp. if its Ct value is defined in the results grid (the fluorescence curve crosses the threshold line) in the JOE/Yellow/HEX channel.
3. The sample is considered to be positive for *Mycoplasma genitalium* if its Ct value is defined in the results grid (the fluorescence curve crosses the threshold line) in the ROX/Orange channel.
4. The sample is considered to be negative for *Chlamydia trachomatis, Ureaplasma* spp. and *Mycoplasma genitalium* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM/Green, JOE/Yellow/HEX and ROX/Orange channels and in the results grid in the Cy5/Red channel the Ct value doesn't exceed boundary value.

* For Ct boundary values of the samples, Negative Control of Extraction and Positive Control of Amplification, see the **Important product information bulletin**.

10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

- If no signal is detected for Positive Control of amplification (C+) or if its Ct value exceeds the boundary Ct value in FAM/Green, JOE/Yellow/HEX and ROX/Orange channels, PCR should be repeated for the samples in which signal was not detected.
- If the positive signal in negative controls (C- or NCA) in the channels for detection of pathogen DNA is registered, analysis should be repeated for the samples in which Ct value was detected.
- If no signal was detected in the channels intended for pathogen DNA and Internal Control detection, the result is considered to be invalid. The sample should be examined once again.

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

11. TRANSPORTATION

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-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® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT** PCR kit

are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium* 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 ºC 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	*Chlamydia trachomatis*	$5x10^2$
			Ureaplasma spp.	10^3
			Mycoplasma genitalium	10^3
Urine [5]	DNA-sorb-AM	PCR kit variants FRT and FRT-100 F	*Chlamydia trachomatis*	10^3
			Ureaplasma spp.	$2x10^3$
			Mycoplasma genitalium	$2x10^3$

The analytical sensitivity of each microorganism does not change even if two other microorganisms are present at high concentrations.

13.2. Specificity

The analytical specificity of **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-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® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT** PCR kit was confirmed in laboratory clinical trials.

Nonspecific responses were absent in tests of human DNA samples and DNA of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Chlamydia trachomatis, Mycoplasma genitalium, Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* types 1 and 2, *CMV,* and *HPV*.

[3] The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.

[4] Urogenital swabs are to be placed into the Transport Medium for Swabs (**REF** 956-CE, 987-CE) or Transport Medium with Mucolytic (**REF** 952-CE, 953-CE).

[5] Treatment is required.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institute of Science “Central Research Institute of 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”, 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® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”