CE 1023

IVD

For Professional Use Only

AmpliSens® *Chlamydia trachomatis*-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***Chlamydia trachomatis*-FEP PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of *Chlamydia trachomatis* DNA in the clinical materials (urogenital, rectal, and throat swabs; eye discharge; urine; and prostate gland secretion) by end-point 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

Chlamydia trachomatis detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome special region using specific primers. In end-point PCR the amplified product is detected via fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in the reaction mixture after PCR. **F**luorescent **E**nd-**P**oint PCR (FEP-PCR) allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens**® ***Chlamydia trachomatis*-FEP** 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 of each individual sample and to identify possible reaction inhibition. **AmpliSens**® ***Chlamydia trachomatis*-FEP** 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. Wax melts and reaction components mix only at 95 ºC.

3. CONTENT

AmpliSens® ***Chlamydia trachomatis*-FEP** PCR kit is produced in 2 forms:

AmpliSens® *Chlamydia trachomatis*-FEP PCR kit (0.5-ml tubes),

REF B1-100-R0,5-FEP-CE.

AmpliSens® *Chlamydia trachomatis*-FEP PCR kit (0.2-ml tubes),

REF B1-100-R0,2-FEP-CE.

AmpliSens® *Chlamydia trachomatis*-FEP PCR kit includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FL *Chlamydia trachomatis* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
PCR-mix-Background-red	red clear liquid	0.6	1 tube
Mineral oil for PCR*	colorless viscous liquid	4.0	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 for thermocyclers without a constant-temperature lid.

** must be used in the extraction procedure as Negative Control of Extraction.

*** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE protocol).

AmpliSens® ***Chlamydia trachomatis*-FEP** PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves.
- 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.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems,USA), Mazygene (Axygen, USA), Terzik (DNA-Technology, Russia).
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR (0.5- 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 filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance 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 and 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 unidirectional; 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

Sampling of biological materials for PCR-analysis, transportation, and storage are described in detail in handbook of the manufacture [1]. It is recommended that this handbook is read before beginning of the work.

AmpliSens® *Chlamydia trachomatis*-FEP PCR kit is intended to analyze DNA extracted with DNA extraction kits from:

- *urogenital, rectal, and throat swabs;*
- *eye discharge;*
- *urine (a sediment of the first portion of the morning specimen);*
- *prostate gland secretion.*

7. WORKING CONDITIONS

AmpliSens® *Chlamydia trachomatis*-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

It is recommended that the following nucleic acid extraction kits are used:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- Other nucleic acid extraction kits recommended by CRIE.

Extract DNA according to the instructions provided by the manufacturer.

8.2. Preparing PCR

Total reaction volume is **30 µl**, 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 *Chlamydia 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 wax layer of each tube, ensuring that it does not fall under the wax and mix with PCR-mix-1-FL *Chlamydia trachomatis*.
3. Add above 1 drop of mineral oil for PCR (~ 25 µl).
4. Prepare one **Background** sample. To do this, mark a tube with **PCR-mix-1-FL *Chlamydia trachomatis*** as **Background** and add **20 µl** of **PCR-mix-Background-red** above the wax layer surface. Ensure that PCR-mix-Background-red does not fall under the wax and mix with PCR-mix-1-FL *Chlamydia trachomatis.* Add above **1** drop of **mineral oil for PCR**.

Use **mineral oil for PCR** if working with thermocyclers without a constant-temperature lid.

Use **PCR-mix-Background-red** solution only if DNA samples were isolated with DNA-sorb-AM or DNA-sorb-B kits. If any other nucleic acid extraction kits (recommended by CRIE) were used, follow the instructions provided by the manufacturer.

5. Using tips with aerosol barrier, add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction.

6. Carry out control 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 a sample extracted from the **Negative Control** to the tube labeled C- (Negative Control of Extraction).

8.2.2 Amplification

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 ºC (pause mode), insert the tubes into the cells of the thermocycler and press the button to continue.

It is recommended that drops are removed from walls of the tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

Table 1

AmpliSens-1-FEP amplification program

	Terzik			**GeneAmp PCR System 2700**			**Gradient Palm Cycler, MaxyGene**		
Step	Tempera ture, °C	Time	Cycles	Tempera ture, °C	Time	Cycles	Tempera ture, °C	Time	Cycles
0	**95**	Pause		**95**	Pause		**95**	Pause	
1	**95**	5 min	1	**95**	5 min	1	**95**	5 min	1
2	**95**	2 s	35	**95**	20 s	20	**95**	2 s	24
	65	5 s		**65**	25 s		**65**	10 s	
	72	5 s		**72**	30 s		**72**	10 s	
3	**95**	2 s	9	**95**	20 s	24	**95**	2 s	20
	60	10 s		**60**	30 s		**60**	15 s	
	72	5 s		**72**	30 s		**72**	10 s	
4	**95**	2 s	1	**95**	20 s	1	**95**	2 s	1
	60	10 s		**60**	30 s		**60**	15 s	
5	**10**	Storage		**10**	Storage		**10**	Storage	

Amplification programs for different thermocycler models are described in **Guidelines** "**End-Point PCR Detection of STIs and Other Reproductive Tract Infections**" [2].

9. DATA ANALYSIS

Detection is performed with florescence detector according to the protocol provided by the manufacturer (please read the Instrument Operating Manual before using this kit).

The fluorescent signal intensity is detected in two channels:

– The signal from the *Chlamydia trachomatis* DNA amplification product is detected in the FAM channel (or analogous, depending on the detector model);

– The signal from the Internal Control amplification product is detected in the HEX channel (or analogous, depending on the detector model).

Prior to detection, all settings should be entered and saved. Refer to the **Guidelines** and the **Important Product Information Bulletin** for settings.

Results interpretation

1. Principle of interpretation:

— *Chlamydia trachomatis* DNA is **detected** in a sample if its signal in the FAM channel is greater than the defined threshold value of the positive result.

— *Chlamydia trachomatis* DNA is **not detected** in a sample if its signal in the FAM channel is less than specified threshold value of the negative result while the signal in the HEX channel is greater than the defined threshold value.

— The result is **invalid** if the signal of a sample in the FAM channel is less than defined threshold of the negative result and the signal in the HEX channel is less than the defined threshold value.

— The result is **equivocal** if the signal of a sample in the FAM channel is greater than the defined threshold value of the negative result but less than the threshold value of the positive result (the signal is between thresholds).

Run the PCR test for the sample once again if the result is **invalid** or **equivocal**.

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 (Table 2).

Table 2

Results for controls

Control	Stage for control	Signal in the channel		Interpretation
		FAM	HEX	
C–	DNA extraction	**<** threshold of negative result	**>** threshold	"–" or OK
NCA	Amplification	**<** threshold of negative result	**<** threshold	"nd"
C+	Amplification	**>** threshold of positive result	**>** threshold	"+" or OK

10. TROUBLESHOOTING

- If the signal of the Positive Control of amplification (C+) in the FAM channel is less than the threshold of the positive result, run PCR and detection for all samples in which *Chlamydia trachomatis* DNA was not found.
- If the signal of the Negative Control of extraction (C-) and/or Negative Control of amplification (NCA) in the FAM channel is more than the threshold of the positive result, run PCR test starting from the extraction for all samples in which *Chlamydia trachomatis* DNA was found.

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

11. TRANSPORTATION

AmpliSens® *Chlamydia trachomatis*-FEP PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Chlamydia trachomatis*-FEP** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *Chlamydia trachomatis*-FEP** 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 *Chlamydia trachomatis* should be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Chlamydia trachomatis*-FEP** PCR kit is specified in the table below.

Clinical material	Transport medium	DNA extraction kit	Analytical sensitivity, GE/ml*
Urogenital swabs	Transport Medium for Swabs or Transport Medium with Mucolytic	DNA-sorb-AM	5×10^2
Urine (pretreatment is required)	–	DNA-sorb-AM	1×10^3

* Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

13.2. Specificity

The analytical specificity of **AmpliSens® *Chlamydia trachomatis*-FEP** PCR kit is ensured by selection of specific primers and probes as well as by selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. Nonspecific responses were absent during examination of human DNA as well as a DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Candida albicans, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Neisseria flava, Neisseria subflava, Neisseria sicca, Neisseria mucosa, Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* type 1 and 2, *CMV,* and *HPV.*

The clinical specificity of **AmpliSens® *Chlamydia trachomatis*-FEP** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institution of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines “End-Point PCR Detection of STIs and Other Reproductive Tract Infections”, developed by Federal Budget Institution 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 accordance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens®**

***Chlamydia trachomatis*-FEP** 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
IVD	*In vitro* diagnostic medical device		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
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institution of Science "Central Research Institute for Epidemiology"	**IC**	Internal control

List of Changes Made in the Instruction Manual

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