



For *in Vitro* Diagnostic Use

TABLE OF CONTENTS

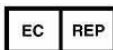
1. INTENDED USE.....	3
2. PRINCIPLE OF PCR DETECTION.....	3
3. CONTENT.....	3
4. ADDITIONAL REQUIREMENTS.....	4
5. GENERAL PRECAUTIONS.....	4
6. SAMPLING AND HANDLING.....	5
7. PROTOCOL.....	5
8. DATA ANALYSIS.....	7
9. TROUBLESHOOTING.....	8
10. STABILITY AND STORAGE.....	8
11. SPECIFICATIONS.....	8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

AmpliSens[®] *Chlamydia trachomatis*-FEP

PCR kit

Instruction Manual

AmpliSens[®]



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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; prostate gland secretion) by means of 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 usually linked to oligonucleotide probes which bind specifically to the amplified product in ordinary thermocycler. Multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in a reaction mixture after PCR. Fluorescent End-Point PCR (FEP-PCR) allows the detection of the accumulating product without re-opening of the reaction tubes after the PCR run. **AmpliSens® Chlamydia trachomatis-FEP PCR kit** is a qualitative test, which contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation of each individual sample and to identify possible reaction inhibition. **AmpliSens® Chlamydia trachomatis-FEP PCR kit** uses “hot-start”, that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Melting of wax and mix of reaction components occur only at 95 °C, which greatly diminish frequency of nonspecifically primed reactions.

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 <i>Chlamydia trachomatis</i> ready-to-use single-dose test tubes (<i>under wax</i>)	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 in the isolation procedure as Negative Control of Extraction.

** add 10 µl of Internal Control during the DNA isolation 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 isolation 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), MAXYGENE (Axygen, USA), Terzik (DNA-Technology, Russia).
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity (for example, Axygen, USA).
- Refrigerator with temperature at 2 – 8 °C.
- Deep-freezer with temperature at or below minus 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 mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, 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 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



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 isolation kits from:

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

7. PROTOCOL

7.1. DNA Isolation

It's 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



Please carry out the DNA isolation according to the instructions provided by the manufacturer.

7.2. Preparing the PCR

Total reaction volume is **30 µl**, volume of DNA sample is **10 µl**.

7.2.1 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** (about **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**.



Apply **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).

7.2.2 Amplification

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 °C (pause regimen), insert the tubes to 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 vortexing (1–3 sec) before placing in the thermocycler.

Table 1 **AmpliSens-1-FEP amplification program**

Step	"Terzik"			"GeneAmp PCR System 2700"			"Gradient Palm Cycler", "MAXYGEN"		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles	Temperature, °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 sec	35	95	20 sec	20	95	2 sec	24
	65	5 sec		65	25 sec		65	10 sec	
	72	5 sec		72	30 sec		72	10 sec	
3	95	2 sec	9	95	20 sec	24	95	2 sec	20
	60	10 sec		60	30 sec		60	15 sec	
	72	5 sec		72	30 sec		72	10 sec	
4	95	2 sec	1	95	20 sec	1	95	2 sec	1
	60	10 sec		60	30 sec		60	15 sec	
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]

8. DATA ANALYSIS

Detection is performed with fluorescence 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

Principle of interpretation:

- *Chlamydia trachomatis* DNA is **detected** in a sample if its signal in the FAM channel is more than specified threshold 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 of the negative result while the signal in the HEX channel is more than specified 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 a signal in the HEX channel is less than specified threshold value as well.

- The result is **equivocal** if the signal of a sample in the FAM channel is more than 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 its result is **invalid** or **equivocal**.

1. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2).

Table 2

Results for controls

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

9. 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 isolation for all samples in which *Chlamydia trachomatis* DNA was found.

10. STABILITY AND STORAGE

All components of the **AmpliSens® Chlamydia trachomatis-FEP** PCR kit are to be stored from 2 °C to 8 °C, when not in use, and are stable until the expiry date stated on the label.



PCR-mix-1-FL *Chlamydia trachomatis* should be kept away from light.

11. SPECIFICATIONS

11.1. Sensitivity

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

Clinical material	Transport medium	DNA isolation kit	Analytical sensitivity
Urogenital swabs	“Transport medium for swabs” or “Transport medium with mucolytic”	“DNA-sorb-AM”	5 x 10 ² GE/ml*
Urine (pretreatment is required)	–	“DNA-sorb-AM”	1 x 10 ³ GE/ml

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

11.2. 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 strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. There were not nonspecific test responses during examination of a 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 1 and 2*, *CMV*, *HPV*.

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
















12. REFERENCES

1. Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science “Central Research Institute of 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” issued by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

13. QUALITY CONTROL

In accordance with Federal State Institution of Science “Central Research Institute of 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.

14. EXPLANATION OF SYMBOLS

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Authorized representative in the European Community
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use		Internal Control
	Negative Control of Amplification		Negative control
	Central Research Institute of Epidemiology, Moscow, Russia		