



For Professional Use Only

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 material (urogenital, rectal, and oropharyngeal swabs; conjunctival 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 special *Chlamydia trachomatis* primers. In Fluorescent End-Point PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after the PCR. It allows the detection of the 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 (Internal Control-FL (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 using a wax layer. Wax melts and reaction components mix only at 95 °C.

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	Quantity
PCR-mix-1-FL <i>Chlamydia trachomatis</i> 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 dropper bottle
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

* is used if DNA samples were extracted using DNA-sorb-AM or DNA-sorb-B kits.

** 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

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- 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)).
- 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 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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge them briefly.
- Use disposable protective gloves, laboratory coats, 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 samples and unused reagents in compliance with the local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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 to 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.

6. SAMPLING AND HANDLING



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

AmpliSens® *Chlamydia trachomatis*-FEP PCR kit is intended for analysis of the DNA extracted with the use of DNA extraction kits from the clinical material (urogenital, rectal, and oropharyngeal swabs; conjunctival 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 to use the following nucleic acid extraction kits:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- For other nucleic acid extraction kits see Guidelines [2].

The DNA extraction of each test sample is carried out in the presence Internal Control-FL (IC).



Extract the DNA according to the manufacturer's protocol.

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis.

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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 the wax layer into 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** (if using thermocyclers without a constant-temperature lid).
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** 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 *Chlamydia trachomatis*. Add above **1** drop of **mineral oil for PCR** (if using thermocyclers without a constant-temperature lid).



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

5. Using filter tips, add **10 µl** of the **DNA samples** obtained at the stage of DNA extraction.

6. Carry out the 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 (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. Run the following program in the thermocycler (see Table 1).

2. When the temperature reaches 95 °C (pause mode), insert tubes into the wells of the thermocycler and press the button to continue.

Table 1

AmpliSens-1-FEP amplification program

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

Note – Amplification programs for some other models of thermocyclers are specified in Guidelines [2].

3. Proceed to fluorescence detection after the amplification program is completed.

9. DATA ANALYSIS

The detection is performed by means of a fluorescence detector (according to the operator manual for the used instrument) by measuring the fluorescence signal intensity in three

channels:

- The FAM channel (or analogous, depending on the detector model) is intended for the detection of the signal of the *Chlamydia trachomatis* DNA amplification product.
- The HEX channel (or analogous, depending on the detector model) is intended for the detection of the signal of the IC DNA amplification product.



Before the detection run, the required settings of the detector software should be adjusted according to the *Important Product Information Bulletin* enclosed to the PCR kit and Guidelines [2].

The obtained results are interpreted on the basis of the level of fluorescence signal in the corresponding channels relatively to the background for the clinical and control samples. Interpretation is performed automatically by the software of the instrument used.

The principle of interpretation is the following:

- *Chlamydia trachomatis* DNA is **detected** if the signal determined in the FAM channel is greater than the specified threshold value of the positive result.
- *Chlamydia trachomatis* DNA is **not detected** if the signal determined in the FAM channel is less than the specified threshold value of the negative result, whereas the signal determined in the HEX channel is greater than the specified threshold value.
- The result is **invalid** if the signal determined in the FAM channel is less than the specified threshold value of the negative result, whereas the signal determined in the HEX channel is less than the specified threshold value.
- The result is **equivocal** if the signal of a sample determined in the FAM channels is greater than the specified threshold value of the negative result but less than the threshold of the positive result (the signal is between the threshold values).



If the result is **invalid** or **equivocal** for the sample, PCR analysis of this clinical sample should be repeated.

The result of the analysis is considered reliable only if the results obtained for the 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	Signal in the channel	
		FAM	HEX
C-	DNA extraction	< threshold value of negative result	> threshold value
NCA	PCR	< threshold value of negative result	< threshold value
C+	PCR	> threshold value of positive result	> threshold value

10. TROUBLESHOOTING

1. If the signal determined for the Positive Control of amplification (C+) in the FAM channel is less than the threshold value of the positive result, the amplification and detection should be repeated for all samples in which *Chlamydia trachomatis* DNA was not detected.
2. If the signal determined for the Negative Control of extraction (C-) and/or Negative Control of amplification (NCA) in the FAM channel is greater than the threshold value of the positive result, the PCR analysis should be repeated starting from the DNA extraction stage for all samples in which *Chlamydia trachomatis* DNA was detected.

If you have any further questions or if you 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 °C 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 °C when not in use. All components of the **AmpliSens® *Chlamydia trachomatis*-FEP** PCR kit are stable until the expiration date stated 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* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Transport medium	DNA extraction kit	Analytical sensitivity, GE/ml ¹
Urogenital swabs	Transport Medium for Swabs REF 987-CE or Transport Medium with Mucolytic Agent REF 953-CE	DNA-sorb-AM	5 x 10 ²
Urine (pretreatment is required)	–	DNA-sorb-AM	1 x 10 ³

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 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 Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines “End-Point PCR Detection of STIs and Other Reproductive Tract Infections”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

¹ Genome equivalents (GE) of the microorganism per 1 ml of a clinical material placed into the specified transport medium.

15. QUALITY CONTROL

In compliance with the Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *Chlamydia trachomatis*-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	<i>In vitro</i> diagnostic medical device		Expiration Date
	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
	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”
14.07.15 ME	Text	Corrections according to the template
	1. Intended use	The clinical material was specified
	6. Sampling and handling	
	3. Content	For the Mineral oil for PCR reagent, tube was changed to dropper bottle. For the PCR-mix-Background-red reagent, the note “is used if DNA samples were extracted using DNA-sorb-AM or DNA-sorb-B kits” was added
13.1. Sensitivity	The catalogue numbers for the transport media were added	