RUO

Research Use Only

AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT PCR kit

Instruction Manual

AmpliSens®

Federal Budget Institute of Science “Central Research Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Chlamydia trachomatis* DNA in clinical materials (urogenital, rectal, oropharyngeal and conjunctival swabs, 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

Qualitative and quantitative detection of *Chlamydia trachomatis* DNA by the polymerase chain reaction (PCR) includes the following stages: (1) *Chlamydia trachomatis* DNA extraction from clinical materials in the presence of Internal Control (IC), which must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition; and (2) real-time PCR amplification of *Chlamydia trachomatis* DNA and IC.

Chlamydia trachomatis detection is based on the amplification of the pathogen genome specific region using specific *Chlamydia trachomatis* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that 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® *Chlamydia trachomatis*-screen-titre-FRT** 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 chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The result of Internal Control amplification is detected in the JOE/Yellow fluorescence channel. The DNA target selected as an endogenous internal control is a fragment of human genome (a β-globin gene fragment). It must be always present in the sample (urogenital swab) in sufficient quantities equivalent to the number of cells in the swab (10^3–10^5 of genome equivalents). Thus, the use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and amplification) but also to assess the adequacy of sampling and storage of clinical material. If epithelial swab was taken incorrectly (the number of epithelial cells is insufficient), the amplification signal of β-globin gene will be underestimated. The number of epithelial cells may be insufficient if

rectal, oropharyngeal, and conjunctival swabs as well as prostate gland secretion and urine samples are used.

Quantitative DNA analysis is based on the linear dependence between the cycle threshold (Ct) and the initial concentration of DNA target. Quantitative analysis is performed in the presence of DNA calibrators (samples with a known concentration of *Chlamydia trachomatis* DNA), which are added during amplification. The results of amplification of DNA calibrators are used to construct a calibration curve, on the basis of which the concentration of *Chlamydia trachomatis* DNA in samples determined. To minimize the effect of variation during material sampling, the quantitative results (*Chlamydia trachomatis* DNA concentrations) are normalized to the genomic DNA quantity.

3. CONTENT

AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT PCR kit is produced in 1 form:

AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT PCR kit variant FRT-100 F (for use with RG, iQ, Mx) **REF** R-B1-100-FT(RG,iQ,Mx)-CE.

AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT PCR kit variant FRT-100 F includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FL *Chlamydia trachomatis*-screen-titre	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
DNA-buffer	colorless clear liquid	0.5	1 tube
DNA calibrator UG1	colorless clear liquid	0.1	1 tube
DNA calibrator UG2	colorless clear liquid	0.1	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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE, DNA-sorb-B, **REF** K1-2-100-CE protocols).

AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT PCR kit 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); Rotor-Gene Q (Qiagen, Germany), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- 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 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 another suitable disinfectant.

- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa 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 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 are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from clinical material (urogenital, rectal, oropharyngeal and conjunctival swabs, prostate gland secretion and urine samples).

7. WORKING CONDITIONS

AmpliSens® *Chlamydia trachomatis*-screen-titre-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.
- DNA-sorb-B, **REF** K1-2-100-CE (for the prostate gland secretion).

Extract DNA according to the manufacturer's instructions.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

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

1. Prepare the reaction mixture straight before the test. Mix reagents for one reaction in the following proportion:
   - **10 µl of PCR-mix-1-FL *Chlamydia trachomatis*-screen-titre,**

— **5 µl of PCR-mix-2-FRT and polymerase (TaqF)** mixture.

2. Before starting work, it is necessary to prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. Transfer the content of one tube with **polymerase (TaqF) (30 µl)** to the tube with **PCR-mix-2-FRT (300 µl)** avoiding foaming. Mark each tube with the mixture preparation date.

The prepared mixture is intended for analysis of 60 samples. The mixture should be stored at 2–8 ºC until use (not longer than for 3 months).

If the mixture cannot be utilized within 3 months, it should be prepared for a smaller number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and **15 µl of polymerase (TaqF).** Thus prepared mixture is intended for 30 reactions.

3. Thaw and vortex the tube with **PCR-mix-1-FL *Chlamydia trachomatis*-screen-titre**. Centrifuge shortly to remove the drops from the caps of the tubes.
4. Calculate the required number of reactions including the test and control samples according to Appendix 1. Note that even for analysis of one test DNA sample in the qualitative format, it is necessary to run 3 controls of the PCR amplification stage: 2 calibrators (UG1 and UG2) and the Negative Control of Amplification (DNA-buffer). It is necessary to take reagents for one extra reaction: for N tests, prepare reagents for (N+1) reactions.
5. Prepare the reaction mixture in an individual tube. Mix **PCR-mix-1-FL *Chlamydia trachomatis*-screen-titre and PCR-mix-2-FRT, and polymerase (TaqF)**, which was prepared as described in point 1 of Section 7.2.1.
6. Prepare the required number of tubes for amplification of DNA from clinical and control samples.
7. Transfer **15 µl** of prepared reaction mixture into the tubes.
8. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes using tips with aerosol barrier.
9. Carry out the control amplification reactions:

NCA	- Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
Calibrators UG1 and UG2	- Add **10 µl** of **UG1** and **10 µl** of **UG2** to the two tubes.
C–	- Add **10 µl** of sample isolated from **Negative Control** to the tube labeled C- (Negative Control of Extraction).

8.2.2 Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

1. Create a temperature profile on your instrument as follows:

AmpliSens-1 amplification program

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	***Temperature, °C***	***Time***	**Cycles**	***Temperature, °C***	***Time***	**Cycles**
Hold	**95**	15 min	1	**95**	15 min	1
Cycling 1	**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 *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

Fluorescent signal is detected at the 2nd step (60°C) of stage Cycling 2 in FAM/Green and JOE/Yellow channels.

2. Adjust the fluorescence channel sensitivity according to **Important Product Information Bulletin**.
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

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

- ***Chlamydia trachomatis* DNA** is detected in the **FAM/Green** fluorescence channel,
- **IC DNA** is detected in the **JOE/Yellow** fluorescence channel.

See the **Manufacturer's manual, Guidelines** and **Important product information bulletin** for data analysis settings.

Interpretation of results

The results of analysis are accepted as relevant if the results obtained for all controls (C-, NCA, UG1, UG2) are correct and the calculated concentration is in the range of concentrations indicated in the **Important Product Information Bulletin.**

Results for controls

Control	Stage for control	Ct in FAM/Green and JOE/Yellow channels	Interpretation
C–	DNA extraction	Neg	OK
NCA	Amplification	Neg	OK
UG1, UG2	Amplification	Ct value and calculated concentration are defined	OK

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[2] For example, iCycler, iQ5, Mx3000P, Mx3000 or equivalent.

1. *Chlamydia trachomatis* DNA is **detected** in a sample if its Ct value is defined in the results grid in the FAM/Green channel.
2. *Chlamydia trachomatis* DNA is **not detected** in a sample if its Ct value is not defined in the results grid in the FAM/Green channel (the fluorescence curve does not cross the threshold line) whereas the Ct value in the JOE/Yellow channel in the results grid is defined and the quantity of human genome equivalents per reaction is greater than 10^3 for women and greater than $5x10^2$ for men.
3. The result of analysis is **invalid** if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/Yellow channel or the number of human genome equivalents per reaction is less than 10^3 for women and less than $5x10^2$ for men. In this case, PCR should be repeated starting from the DNA extraction.

The DNA concentration per human genome equivalents is calculated by the following formula:

$$\log\left[\frac{\textit{C.trachomatis}\ \text{DNA copies}}{\text{Human DNA copies}} \times 200000\right] = \log\left[\textit{C.trachomatis}\ \text{per}\ 10^5\ \text{of cells}\right]$$

The concentrations of DNA calibrators are specified in the **Important Product Information Bulletin.**

In case of using rectal, oropharyngeal, and conjunctival swabs as well as prostate gland secretion and urine samples, human genome equivalents quantity per reaction can be less than 10^3 for women and less than $5x10^2$ for men.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If the Ct value is absent in both JOE/Yellow and FAM/Green channels or the Ct value in the JOE/Yellow /HEX channel is higher than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (if iCycler iQ or iQ5 instruments are used).
2. If the Ct value is present for C– and/or for NCA in the FAM/Green channel in the results grid. In this case, it is necessary to repeat PCR testing for all samples with *Ct* determined in FAM/Green.
3. If a Calc Conc (copies/reaction) value greater than 5 appears in the results grid for the negative control of extraction (C–) and/or amplification (NCA) in the JOE/Yellow channel, it indicates contamination of reagents or samples. In such cases the results of analysis are considered invalid. Test analysis must be repeated (beginning with DNA extraction

stage) for those samples that have a signal in the FAM/Green channel and measures to detect and eliminate the source of contamination must be taken.

4. If the Ct value for DNA calibrators (UG1 and UG2) is not detected in the FAM/Green and/or JOE/Yellow channels, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
5. If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

11. TRANSPORTATION

AmpliSens® *Chlamydia trachomatis*-screen-titre-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® *Chlamydia trachomatis*-screen-titre-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Chlamydia trachomatis*-screen-titre-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.

Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FL *Chlamydia trachomatis*-screen-titre is to be kept away from light.

13. SPECIFICATIONS

13.1 Sensitivity

The analytical sensitivity of **AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT** PCR kit is the following:

Clinical material	Transport Medium	Nucleic acid extraction kit	Sensitivity, GE/ml [3]
Urogenital swabs[4]	Transport Medium for Swabs, Transport Medium with Mucolytic Agent	DNA-sorb-AM	$5x10^2$
Urine[5]	–	DNA-sorb-AM	$1x10^3$

13.2 Specificity

The analytical specificity of **AmpliSens® *Chlamydia trachomatis*-screen-titre-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 deposited in gene banks by sequence comparison analysis. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Mycoplasma genitalium, Neisseria gonorrhoeae, Neisseria* spp., *Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma hominis, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* types 1 and 2, *CMV,* and *HPV.* The clinical specificity of **AmpliSens® *Chlamydia trachomatis*-screen-titre-FRT** 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, 2008.
2. Guidelines “Real-Time PCR Detection of STIs and Other Reproductive Tract Infections (screen-titres)”, 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.

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

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

[5] Treatment is required.

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®** ***Chlamydia trachomatis*-screen-titre-FRT** 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	Σ	Sufficient for
RUO	Research use only		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
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**UG1, UG2**	DNA calibrators
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
07.12.10	Cover page	The phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
02.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
24.08.12 lvl	10. Troubleshooting	Mention of JOE/Yellow channel was eliminated from the paragraph 2
		Paragraph 3 containing information about criteria of contamination of reagents and samples was added
	Title page, Key to symbols used	Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only
31.05.13 FN	Cover page	The phrase “For Professional Use Only” was changed to “Research use only”
	Specifications, Sensitivity	The information about Transport Medium for Swabs and Transport Medium with Mucolytic Agent was added to the table describing analytical sensitivity