CE

IVD

For Professional Use Only

AmpliSens® *Treponema pallidum*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***Treponema pallidum*****-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Treponema pallidum* DNA in clinical materials (urogenital, rectal, and oral swabs; blister exudate; and discharge of erosive-ulcer lesions of human skin and mucous membranes) 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

Treponema pallidum detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Treponema pallidum* 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 fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***Treponema pallidum*****-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®** ***Treponema pallidum*****-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 wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C. The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® ***Treponema pallidum*****-FRT** PCR kit is produced in 3 forms:

AmpliSens® *Treponema pallidum*-FRT PCR kit variant FRT (for use with RG),

REF R-B20(RG)-CE.

AmpliSens® *Treponema pallidum*-FRT PCR kit variant FRT (for use with iQ),

REF R-B20(iQ)-CE.

AmpliSens® *Treponema pallidum* -FRT PCR kit variant FRT-100 F (for use with RG, iQ),

REF R-B20-F(RG,iQ)-CE.

AmpliSens® *Treponema pallidum*-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *Treponema pallidum* (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	colorless 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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE protocol).

AmpliSens® *Treponema pallidum*-FRT PCR kit is intended for 110 reactions (including controls).

AmpliSens® *Treponema pallidum*-FRT PCR kit variant FRT-100 F includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *Treponema pallidum*	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 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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE protocol).

AmpliSens® *Treponema pallidum*-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 a rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iCycler iQ or iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA), DT-96 (DNA-Technology, Russia) 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, or 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 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

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® *Treponema pallidum*-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from urogenital, rectal, and oral swabs; blister exudate; and discharge of erosive-ulcer lesions of human skin and mucous membranes.

7. WORKING CONDITIONS

AmpliSens® *Treponema pallidum*-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.

Carry out the DNA extraction according to the manufacturer's instructions.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

Variant FRT

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

1. Prepare the required number of tubes with **PCR-mix-1-FL *Treponema pallidum*** 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 *Treponema pallidum***.
3. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage into the prepared tubes using tips with aerosol barrier.
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).

Variant FRT-100F

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

1. Thaw the **PCR-mix-2-FRT** tube. Vortex the tubes with **PCR-mix-1-FL *Treponema pallidum*, PCR-mix-2-FRT, and polymerase (TaqF)** then centrifuge briefly.
2. For N reactions (including 2 controls) add in a new tube:

   10*(N+1) µl of **PCR-mix-1-FL *Treponema pallidum***,

   5.0*(N+1) µl of **PCR-mix-2-FRT**,

   0.5*(N+1) µl of **polymerase (TaqF)**.
3. Transfer **15 µl** of the prepared mixture to the prepared tubes.
4. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage into the prepared tubes using tips with aerosol barrier.
5. 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 real-time amplification instrument according to manufacturer's manual and Guidelines [2].

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

Table 1

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	**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 in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores on the 2nd step (60 °C) of stage Cycling 2 (other channels are enabled if several tests are simultaneously carried out in a single run).

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.

AmpliSens-1 RG general program allows simultaneous conducting of any combination of tests for detection of DNA of sexually transmitted infection pathogens including tests for identification of *Treponema pallidum* by means of AmpliSens *Treponema pallidum*-FRT PCR kit.

If “multiprime”-format tests for detection of sexually transmitted infections (AmpliSens PCR kits) are carried out simultaneously, the program and template corresponding to the “multiprime” tests should be used.

9. DATA ANALYSIS

IC is detected in the JOE/Yellow/HEX fluorescence channel, *Treponema pallidum* DNA is detected in the FAM/Green fluorescence channel.

See **Guidelines** for data analysis settings for the instrument.

[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.

9.1. Interpretation of results

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

Table 2

Results for controls

Control	Stage for control	Ct value in channel		Interpretation
		FAM/Green	JOE/Yellow/HEX	
C–	DNA extraction	Neg	Pos (< boundary value*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value*)	Pos (< boundary value*)	OK

* For boundary Ct values, see the Important product information bulletin.

1. The sample is considered to be positive for *Treponema pallidum* if its Ct value is determined in the results grid in the FAM/Green channel.
2. The sample is considered to be negative for *Treponema pallidum* if its Ct value is not determined in the results grid (the fluorescence curve does not cross the threshold line) in the FAM/Green channel and if the Ct value determined in the results grid in the JOE/Yellow/HEX channel does not exceed the specified boundary value.
3. The result is considered to be invalid if the Ct value of a sample in the FAM channel is absent and the Ct value in the JOE/Yellow/HEX channel is either absent or more than the specified boundary value. It is necessary to repeat the PCR test for such a sample.

The result of the analysis is 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.

10. TROUBLESHOOTING

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

- If the Ct value is absent in both JOE/Yellow/HEX 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 (for iCycler iQ or iQ5 instruments).
- If the Ct value is present for C– in the FAM/Green channel and/or for NCA in the FAM, JOE/Yellow /HEX channels in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test

analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.

- If no signal is detected for the Positive Controls of Amplification, 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 has not complied 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.
- 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 Cycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® *Treponema pallidum*-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® *Treponema pallidum*-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® *Treponema pallidum*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, 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 *Treponema pallidum* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml [3]
Urogenital swabs	Transport Medium for Swabs (**REF** 956-CE, **REF** 987-CE) or Transport Medium with Mucolytic (**REF** 952-CE, **REF** 953-CE)	DNA-sorb-AM	$1x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens®** ***Treponema pallidum*-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. The clinical specificity of **AmpliSens®** ***Treponema pallidum*-FRT** PCR kit was confirmed in laboratory clinical trials.

Nonspecific responses were absent while testing human DNA samples and DNA samples of 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, Chlamydia trachomatis, Trichomonas vaginalis, Toxoplasma gondiii, HSV* types 1 and 2, *CMV,* and *HPV*.

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

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®** ***Treponema pallidum*-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
29.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”