CE

IVD

For Professional Use Only

AmpliSens® *Treponema pallidum*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

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

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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 the clinical material (urogenital, rectal, and oral swabs; blister exudate; and discharge of erosive-ulcer lesions of human skin and mucous membranes) using real-time 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

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 the real-time PCR, the amplified product is detected with the use of 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 (Internal Control-FL (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. In variant FRT, “hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 °C. In variant FRT-100 F, “hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). 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 2 forms:

AmpliSens® *Treponema pallidum*-FRT PCR kit variant FRT, **REF** R-B20(RG)-CE.

AmpliSens® *Treponema pallidum*-FRT PCR kit variant FRT-100 F, **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)*)	clear liquid from colorless to light lilac colour	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	red 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 (IC)** 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*	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
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 (IC)** 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

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).

- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene tubes when working with PCR kit variant FRT-100 F :
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed caps if a plate-type instrument is used;
  b) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- 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 filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance 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 in 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 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 analysis of DNA extracted by DNA extraction kits from the clinical material (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.

– For other nucleic acid extraction kits see Guidelines [2].

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

In the extraction procedure it is necessary to carry out the control reactions as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labeled C–.

Extract DNA according to the manufacturer's protocols.

8.2. Preparing PCR

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.

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 into each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *Treponema pallidum*.**

Variant FRT-100F

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

1. Thaw the tube with **PCR-mix-2-FRT**. Vortex the tubes with **PCR-mix-1-FL *Treponema pallidum*, PCR-mix-2-FRT, and polymerase (TaqF)** and sediment the drops by short centrifugation (1-2 s).

   Take the required quantity of tubes/ strips for amplification of DNA from clinical and control samples.
2. For N reactions (including 2 controls of amplification) add to 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)**.

   Mix the prepared mixture and sediment the drops by short centrifugation (1-2 s).

   Transfer **15 µl** of the prepared mixture into each tube.

Steps 3 and 4 are required out in both variants.

3. Add **10 µl** of **DNA** obtained at the DNA extraction stage into the prepared tubes.
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(C+)** to the tube labeled C+ (Positive Control of Amplification).

C– – Add **10 µl** of sample extracted from **Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).

8.2.2. Amplification

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***
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**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 for the FAM and JOE fluorophores. 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.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

- The signal of the *Treponema pallidum* DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the IC amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- *Treponema pallidum* DNA is **detected** in a sample if the *Ct* value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of exponential growth of fluorescence.

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

– *Treponema pallidum* DNA is **not detected** if its *Ct* value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channel for the FAM fluorophore, whereas the *Ct* value determined in the results grid in the channel for the JOE fluorophore does not exceed the specified boundary value.
– The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the FAM fluorophore, whereas the *Ct* value in the channel for the JOE fluorophore is not determined (absent) or exceeds specified boundary value. In such case, PCR should be repeated for this sample.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2].

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 (see Table 2).

Table 2

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**	
		FAM	**JOE**
C–	DNA extraction	Absent	< boundary value
NCA	PCR	Absent	Absent
C+	PCR	< boundary value	< boundary value

10. TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of amplification (C+) in the channel for the FAM fluorophore is greater than the specified boundary value or absent. The amplification should be repeated for all the samples in which the *Treponema pallidum* DNA was not detected.
2. The *Ct* value is determined for the Negative Control of Extraction (C–) and/or the Negative Control of Amplification (NCA) in the channel for the FAM fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Treponema pallidum* 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® *Treponema pallidum*-FRT PCR kit should be transported at 2–8 ºС for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Treponema pallidum*-FRT** PCR kit are to be stored at 2–8 °С when not in use (except for polymerase (TaqF) and PCR-mix-2-FRT). All components of the **AmpliSens® *Treponema pallidum*-FRT** PCR kit are stable until the expiry date stated 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 °С.

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 Agent (**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 the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published 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 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 gondii, HSV* types 1 and 2, *CMV,* and *HPV*.

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

The clinical specificity of **AmpliSens®** ***Treponema pallidum*-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, 2010.
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”.

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		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”
30.11.15 ME	Text	Corrections according to the template
	8.1. DNA extraction	Information about controls of extraction was added
	8.2.1. Preparing tubes for PCR	
	9. Data analysis	The sections was rewritten
	10. Troubleshooting	
23.01.18 PM	3. Content	The colour of the reagent was specified
15.03.18 PM	Footer, 3. Content	**REF** R-B20(iQ)-CE was deleted