



For *in Vitro* Diagnostic Use

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AmpliSens® *Treponema pallidum*-EPh

PCR kit

Instruction Manual

AmpliSens®



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1. INTENDED USE.

AmpliSens® *Treponema pallidum*-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Treponema pallidum* in the clinical material (serous exudate of vesicles, ulcers, or erosions; cervical, urethral, or oral scrapes (swabs)) by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION.

Treponema pallidum detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using specific *Treponema pallidum* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *Treponema pallidum*-EPh PCR kit** is a qualitative test and contains the IC which must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *Treponema pallidum*-EPh PCR kit** uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer or chemically modified polymerase (TaqF). Wax melting and reaction mix components occur only at 95°C.

3. CONTENT.

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

AmpliSens® *Treponema pallidum*-EPh PCR kit variant 100 R (vials 0.5 ml),

REF B20-100-R0,5-CE.

AmpliSens® *Treponema pallidum*-EPh PCR kit variant 100 R (vials 0.2 ml),

REF B20-100-R0,2-CE.

AmpliSens® *Treponema pallidum*-EPh PCR kit variant 200.

AmpliSens® *Treponema pallidum*-EPh PCR kit variant 100 R or variant 200 includes:

Reagent	Description	variant 100 R		variant 200	
		Volume (ml)	Amount	Volume (ml)	Amount
PCR-mix-1-R <i>Treponema pallidum</i> ready-to-use single-dose test tubes (<i>under wax</i>)	colorless, clear liquid	0.005	110 tubes of 0.5 or 0.2 ml	---	---
PCR-mix-1 <i>Treponema pallidum</i>	colorless, clear liquid	---	---	1.1	1 tube
PCR-mix-2 red	clear liquid of red color	1.2	1 tube	1.2	2 tubes
Wax for PCR	hard white matter	---	---	1.7	2 tubes
Mineral oil for PCR	colorless viscous liquid	4.0	1 tube	8.0	1 tube
Positive Control DNA <i>Treponema pallidum</i> (C+)	colorless, clear liquid	0.2	1 tube	0.2	1 tube

DNA-buffer	colorless, clear liquid	0.5	1 tube	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube	1.2	1 tube
Internal Control complex ICc**	colorless, clear liquid	1.0	1 tube	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** K1-7-100-CE protocol).

AmpliSens® *Treponema pallidum*-EPh PCR kit variant 100 R is intended for 110 reactions, including controls.

AmpliSens® *Treponema pallidum*-EPh PCR kit variant 200 is intended for 220 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- (DNA isolation kit).
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- PCR box.
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at temperature between 25 °C and 100 °C.
- Tube racks.
- Personal thermocyclers (for example, “Gradient Palm Cycler” (Corbett Research, Australia), “GeneAmp PCR System 2700” (Applied Biosystems), “Biometra”, “MiniCycler”, “PTC-100” (MJ Research)).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature below minus16 °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 other 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 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.



In detail, sampling of biological materials for PCR-analysis, transportation and storage are described in handbook of the manufacture [2]. It is recommended reading this handbook before beginning of the work.

AmpliSens® *Treponema pallidum*-EPh PCR kit is intended to analyze DNA extracted with DNA isolation kits from:

- *Cervical, urethral, or oral scrapes (swabs)*
- *Serous exudate of vesicles, ulcers, or erosions*

7. PROTOCOL.

7.1. DNA Isolation

It's recommended that following nucleic acid extraction kit is used:

- "DNA-sorb-AM", **REF** K1-7-100-CE.



Please carry out the DNA isolation according to the manufacturer instruction.

7.2. Preparing the PCR.

Total reaction volume - 25 µl, volume of DNA sample - 10 µl.

7.2.1 Preparing tubes for PCR.



When using AmpliSens® *Treponema pallidum*-EPh PCR kit variant 100 R steps 1 and 2 should be omitted.

1. Place the tube with **Wax for PCR** into the heat block at 95 °C to melt the wax completely.
2. Prepare required number of the PCR tubes. Pipette 5 µl of **PCR-mix-1 *Treponema pallidum*** into the bottom of each tube. Add a drop (about 10-15 µl) of melted wax above, so it covers completely the liquid, close the caps and mark each tube. The prepared tubes could be stored from 2 to 8 °C during 1 week.
3. Collect the required number of tubes prepared as describes above or tubes with **PCR-mix-1-R *Treponema pallidum*** with wax for amplification of DNA from clinical and control samples.
4. Add **10 µl of PCR-mix-2 red** to the surface of wax layer of each tube, so that it does not fall under the wax and mix with reagents in the tube.
5. Add above 1 drop of **mineral oil for PCR** (about **25 µl**). When using thermocycler with heating cover this step could be omitted.

7.2.2 Amplification.

Use prepared tubes for PCR. Under or immediately above the level of oil, using tips with aerosol barrier, **add 10 µl of DNA samples** obtained from clinical or control samples at the stage of DNA extraction.

Carry out **control amplification reactions**:

- NCA - Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
- C+ - Add 10 µl of **Positive Control DNA *Treponema pallidum*** to the tube for Positive Control of Amplification.

Run the following program on the thermocycler (see table 1). When the temperature will reach 95°C (pause regimen), insert tubes to cells of amplifier and press button to continue.

It is recommended that drops are sedimented from walls of tubes by short vortex (1–3 sec) before their insertion in a thermocycler.

Table 1

Programming thermocyclers for *Treponema pallidum* DNA amplification

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems), "Gradient Palm Cycler" (Corbett Research)			"Biometra", "MiniCycler", "PTC-100" (MJ Research)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	95 °C	15 sec	42	95 °C	1 min	42
	65 °C	10 sec		65 °C	25 sec		65 °C	1 min	
	72 °C	10 sec		72 °C	25 sec		72 °C	1 min	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	4 °C	storage		4 °C	storage		10 °C	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment – 1 h 50 min.


After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored at room temperature for 16 h, between 2 and 8 °C 1 for a week, and below minus 16° for a long time (be sure to warm the samples to room temperature before running electrophoresis).

8. DATA ANALYSIS.

We recommend the following detection agarose kit:

- "EPh" variant 200  K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%). The length of specific amplified DNA fragments is:

- *Treponema pallidum* - 273 bp
- Internal Control - 668 bp



Put the protective mask or use the glass filter while watching and photographing the gel

8.1. Results interpretation

Table 2

Results for controls

Control	Controlled step	Specific bands in the agarose gel		Interpretation
		273 bp	668 bp	
C-	DNA isolation	No	Yes	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	No	OK

- The sample is considered positive for *Treponema pallidum* DNA if the band of 273 bp is present in

agarose gel. The band of IC (668 bp) could be absent in the samples with high concentration of *Treponema pallidum* DNA.

- The sample is considered negative for *Treponema pallidum* DNA if the band of 273 bp is absent and the band of 668 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than 100 bp level of nucleotide pairs.

9. TROUBLESHOOTING.

Analysis results are not obtained as per the following examples:

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lanes none of bands of 273 and 668 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lines nonspecific bands at different levels are present, it may be caused by lack of "hot start" or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C-) specific band of 273 bp appears, it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

10. STABILITY AND STORAGE.

All components of the AmpliSens® *Treponema pallidum*-EPh PCR kit should be stored from 2°C to 8°C when not in use. All components are stable until the expiry date stated on the label.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of AmpliSens® *Treponema pallidum*-EPh PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of AmpliSens® *Treponema pallidum*-EPh PCR kit are guaranteed only when additional reagents kits, "DNA-sorb-AM" and "EPh", are used.

11.2. Specificity.

Specificity of AmpliSens® *Treponema pallidum*-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

12. REFERENCES.

1. Rodionova EN, Gushchin AE, Shipulin GA, Khludova NA, Toporovskii LM, Nikolenko IuA, Toma VS, Dudareva LA, Pokrovskii VV. Detection of *Treponema pallidum* DNA and RNA in clinical material from patients with syphilis at different stages. Zh. Mikrobiol. Epidemiol. Immunobiol. 2003 May-Jun;(3):43-50.
2. 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.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Total Quality Management System, each lot of **AmpliSens® *Treponema pallidum*-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



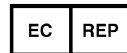
Catalogue number

ICc

Internal Control complex



Contains sufficient for <n> tests



Authorised representative in the European Community.



Consult instructions for use



Caution, consult accompanying documents