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For Professional Use Only

AmpliSens® *Mycoplasma genitalium*-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Mycoplasma genitalium* in the clinical material (cervical or urethral scrapes (swabs), urine sediment, secret of the prostate gland) by means of detection of the amplified products by agarose gel electrophoresis.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Mycoplasma genitalium detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of DNA of pathogen genome using specific *Mycoplasma genitalium* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *Mycoplasma genitalium*-EPh PCR kit** is qualitative test and contains the IC which must be used in the extraction procedure in order to control the extraction process of each individual specimen and to identify possible reaction inhibition.

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit uses “hot-start”, that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Melting of wax and mix of reaction components occur only at 95 ºC, which greatly diminish frequency of nonspecifically primered reactions.

3. CONTENT

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit is produced in 2 forms:

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit variant 100 R (tubes 0.5 ml), **REF** B4-100-R0,5-CE.

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit variant 100 R (tubes 0.2 ml), **REF** B4-100-R0,2-CE.

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit variant 100 R includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix -1-R *Mycoplasma genitalium* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.005	110 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	blue clear liquid	1.2	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Positive Control DNA *Mycoplasma genitalium* (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 complex ICc**	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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE or DNA-sorb-B, **REF** K1-2-100-CE protocols).

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit variant 100 R is sufficient for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves
- DNA extraction kit
- Detection agarose kit
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters (up to 200 µl)
- 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), Uno II (Biometra), MiniCycler, PTC-100 (MJ Research), Terzik (DNA-Technology, Russia);
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for instance, Axygen, USA)**.**
- Refrigerator for 2–8 °C with deep-freezer for ≤ –16 °C.
- Reservoir for disposed tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and put the new tip for every procedure.
- Store and handle amplicons separately from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

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 biological materials for PCR-analysis, transportation and storage is described in handbook of the manufacture [2]. It is recommended to read this handbook before beginning of the work.

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit is intended to analyze DNA extracted with DNA extraction kits from:

- *Cervical or urethral scrapes (swabs)*
- *Urine sediment (use the first part of the stream)*
- *Secret of the prostate gland*

6.1. *Cervical or urethral scrapes (swabs),* obtained with universal probe or cervical brush should be placed into the tube with special transport media (the transport media of the manufacturer is recommended). Break the effective part of the probe with the sample off in the place of the scratch and leave it in the tube. Transfer 0.5 ml of the sample into Eppendorf tube using the tip with aerosol filter. Spin the Eppendorf tube for 5 min at 10,000 g. Discard 0.4 ml of supernatant and stir the pellet in the rest of liquid.

6.2. *Urine sediment* (use the first part of the stream). The first part of the stream (15-25 ml) should be placed into special clean dry vial. Shake the vial and transfer 1 ml of urine into Eppendorf tube using the tip with aerosol filter. Spin the Eppendorf tube for 5 min at 10,000 g. If there are a lot of salts in the sample, then only the upper part of the pellet has to be used. Resuspend it in 1 ml and then concentrate one more time. Discard the supernatant thoroughly. Add the needed volume of transport media to the pellet up to final volume of 0.2 ml. Stir the pellet.

6.3. *Secret of the prostate gland* (0.5-1 ml) should be placed into special clean dry Eppendorf tube. Close the cap and mark the sample.

Only one freeze-thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

Different manufacturers offer DNA extraction kits. We recommend following nucleic acid extraction kits:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- DNA-sorb-B (for secrete of the prostate gland), **REF** K1-2-100-CE.

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

8.2. Preparing the PCR

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

8.2.1 Preparing tubes for PCR

1. Collect the required quantity of the tubes prepared as describes above or tubes with **PCR-mix-1-R *Mycoplasma genitalium*** with wax for amplification of DNA of study and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, so that it wouldn't fall under the wax and mix with reagents in the tube.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.

8.2.2 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.

Perform **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 *Mycoplasma genitalium*** to the tube for Positive Control of Amplification.

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

It is recommended to sediment drops from walls of tubes by short vortex (1–3 s) before their insertion in thermocycler.

Table 1.

Programming thermocyclers at DNA amplification of *Mycoplasma genitalium*

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)**			**Uno II (Biometra), MiniCycler, PTC-100 (MJ Research)**		
Step	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 s	42	**95 °C**	15 s	42	**95 °C**	1 min	42
	65 °C	10 s		**65 °C**	25 s		**65 °C**	1 min	
	72 °C	10 s		**72 °C**	25 s		**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 for 16 h at room temperature, for 1 week at 2-8 °C and for a long time at minus 16 °C (be sure to warm the samples to room temperature before running electrophoresis).

9. DATA ANALYSIS

We recommend the following detection agarose kit:

- EPh variant 200, **REF** 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:

- *Mycoplasma genitalium* - 280 bp
- Internal Control - 550 bp

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

9.1. Results interpretation

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel 280 bp	Specific bands in the agarose gel 550 bp	Interpretation
C-	DNA extraction	No	Yes	Valid result
NCA	Amplification	No	No	Valid result
C+	Amplification	Yes	No	Valid result

- The sample is considered to be positive for *Mycoplasma genitalium* DNA if the band of 280 bp is present in agarose gel. The band of IC (550 bp) could be absent in the samples with high concentration of *Mycoplasma genitalium* DNA.
- The sample is considered to be negative for *Mycoplasma genitalium* DNA if the band of 280 bp is absent and the band of 550 bp is present.

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

10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

- 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 280 and 550 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 presented, 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 280 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.

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

11. TRANSPORTATION

AmpliSens® *Mycoplasma genitalium*-EPh PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

The all components of the AmpliSens® *Mycoplasma genitalium*-EPh PCR kit should be stored at 2–8 ºC and 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.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of AmpliSens® *Mycoplasma genitalium*-EPh PCR kit is no less than $5x10^3$ genome equivalents per 1 ml of sample (GE/ml).

Claimed analytical features of AmpliSens® *Mycoplasma genitalium*-EPh PCR kit are guaranteed only when additional reagents kits, DNA-sorb-AM or DNA-sorb-B (for secret of the prostate gland) and EPh, are used.

13.2. Specificity

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

14. REFERENCES

1. Shipitsyna E, Zolotoverkhaya E, Dohn B, Benkovich A, Savicheva A, Sokolovsky E, Jensen JS, Domeika M, Unemo M. First evaluation of polymerase chain reaction assays used for diagnosis of Mycoplasma genitalium in Russia.J. Eur. Acad. Dermatol. Venereol. 2009 Apr 24.

2. Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal Budget Institution of Science “Central Research Institute for Epidemiology”, Moscow, 2008.

15. QUALITY CONTROL

In accordance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of AmpliSens® *Mycoplasma genitalium*-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	**NCA**	Negative control of amplification
	Upper limit of temperature	**C–**	Negative control of extraction
	Manufacturer	**C+**	Positive control of amplification
	Date of manufacture	**ICc**	Internal Control complex
EC REP	Authorised representative in the European Community		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
12.11.10	Through the text	Records about PCR kit variant 200 are deleted
25.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” 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
24.06.11 VV	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”