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IVD

For Professional Use Only

AmpliSens® *Ureaplasma* spp.-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *Ureaplasma* spp.-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Ureaplasma* species (*U.parvum* and *U.urealiticum*) DNA in the clinical material (cervical or urethral scrapes (swabs), urine sediment, secrete of the prostate gland) using electrophoretic detection of the amplified products in agarose gel.

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

2. PRINCIPLE OF PCR DETECTION

Ureaplasma spp. detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Ureaplasma* spp. primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *Ureaplasma* spp.-EPh** PCR kit is a qualitative test, which contain 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® *Ureaplasma* spp.-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. The wax melting and reaction mix component occurs only at 95 ºC.

3. CONTENT

AmpliSens® *Ureaplasma* spp.-EPh PCR kit is produced in 2 forms:

AmpliSens® *Ureaplasma* spp.-EPh PCR kit variant 100 R (0.5 ml tubes), **REF** B2-100-R0,5-CE.

AmpliSens® *Ureaplasma* spp.-EPh PCR kit variant 100 R (0.2 ml tubes), **REF** B2-100-R0,2-CE.

AmpliSens® *Ureaplasma* spp.-EPh PCR kit variant 100 R includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix -1-R *Ureaplasma* spp.- 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 *Ureaplasma* spp. (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 DNA 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® *Ureaplasma* spp.-EPh PCR kit variant 100 R is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit
- Detection agarose kit
- Disposable powder-free gloves and laboratory coat
- 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), Biometra, MiniCycler, PTC-100 (MJ Research, Terzik (DNA-Technology)
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (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 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

Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [2]. It is recommended to read this handbook before starting work

AmpliSens® *Ureaplasma* spp.-EPh PCR kit is intended to analyze DNA extracted by 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 by universal probe or cervical brush should be placed into the tube with special transport media (the transport media of the manufacturer is recommended). The working part of cytological brushes is to be broken off and left in the tube with transport media. Transfer 0.5 ml of the sample into Eppendorf tube using the tip with aerosol barrier. Spin the Eppendorf tube for 5 min at 10000 g. Remove 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 barrier. Spin the Eppendorf tube for 5 min at 10000 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. Remove the supernatant thoroughly. Add the pellet the needed volume of transport media to the 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® *Ureaplasma* spp.-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

It's recommended to use 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.

Carry out the DNA extraction according to the manufacturer's 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 number of tubes prepared as described above or tubes with **PCR-mix-1-R *Ureaplasma* spp.** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, ensuring that it does not 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

Using tips with aerosol barrier **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction under oil or directly on it.

Carry out the **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 *Ureaplasma* spp.** to the tube for Positive Control of Amplification.

Run the following program on the thermocycler (see table 1). When the temperature reaches 95 ºC (pause regimen), insert tubes into 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 *Ureaplasma* spp.

	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)**		
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 ≤ –16 °C (it's necessary to heat the samples to room temperature before electrophoresis running).

9. DATA ANALYSIS

It's recommended to use 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:

- *Ureaplasma* spp. - 450 bp
- Internal Control - 750 bp

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

Results interpretation

Table 2

Results for controls

Control	**Which step of test is controlled**	**Specific bands in the agarose gel**		**Interpretation**
		450 bp	**750 bp**	
C-	DNA extraction	No	**Yes**	OK
NCA	Amplification	No	No	OK
C+	Amplification	**Yes**	No	OK

- The sample is considered to be positive for *Ureaplasma* spp. DNA if the band of 450 bp is present in agarose gel. The band of IC (750 bp) could be absent in the samples with high concentration of *Ureaplasma* spp. DNA.
- The sample is considered to be negative for *Ureaplasma* spp. DNA if the band of 450 bp is absent and the band of 750 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 100 bp of nucleotide pairs.

10. TROUBLESHOOTING

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

- If the controls analysis results do not correspond to the listed above (Table 2), then the tests are to be repeated. Remove any reagents that may be suspect.
- If in lanes none of bands of 450 and 750 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 material preparing that provoked loss of RNA/DNA or inhibition of 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 specific band of 450 bp appears in lanes corresponding to negative control (NCA, C–) 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® *Ureaplasma* spp.-EPh PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Ureaplasma* spp.-EPh** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *Ureaplasma* spp.-EPh** PCR kit are to be stable until labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of AmpliSens® *Ureaplasma* spp.-EPh PCR kit is no less than $5x10^3$ colour changing units per 1 ml of sample (CCU/ml).

The claimed analytical features of **AmpliSens® *Ureaplasma* spp.-EPh** PCR kit are guaranteed only when additional kits of reagents, DNA-sorb-AM or DNA-sorb-B and EPh (manufactured by Federal Budget Institution of Science "Central Research Institute for Epidemiology") are used.

13.2. Specificity

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

14. REFERENCES

1. Colaizy TT, Kuforiji T, Sklar RS, Pillers DA. PCR methods in clinical investigations of human ureaplasmas: a minireview. Mol. Genet. Metab. 2003 Dec; 80(4):389-97.

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® *Ureaplasma* spp.-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	**IC**	Internal control
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	7.2.1 Preparing tubes for PCR	Items 1 and 2 are deleted
24.12.10 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
27.06.11 VV	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”