CE

IVD

For Professional Use Only

AmpliSens® *Neisseria gonorrhoeae*-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Neisseria gonorrhoeae* DNA in the clinical material (cervical or urethral swabs, conjunctival swabs, urine sediment, and prostate gland secretion) by 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

Neisseria gonorrhoeae detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of DNA of pathogen genome using specific *Neisseria gonorrhoeae* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit** is a qualitative test, which contain the Internal Control (IC). It must be used in the extraction process in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *Neisseria gonorrhoeae*-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. Wax melts and reaction mix components mix only at 95 ºC.

Two pairs of primers are used for detection of *Neisseria gonorrhoeae* DNA:

- *Neisseria gonorrhoeae* 1 – for PCR amplification of cryptic plasmid DNA (395 bp);
- *Neisseria gonorrhoeae* 2 – for PCR amplification of aminoacetyltransferase gene (450 bp).

The test is carried out in two stages. Amplification with *Neisseria gonorrhoeae* 1 pair of primers should be performed at first (screening). Then the samples with positive result should be amplified with *Neisseria gonorrhoeae* 2 pair of primers (confirming).

The samples are considered to be positive for *Neisseria gonorrhoeae* DNA if positive results are detected in both reactions. If positive result is registered in one reaction only, the sample is infected by different species of *Neisseria.*

3. CONTENT

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit is produced in 4 forms:

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit variant 100 R (tubes 0.2 ml) **REF** B5-100-R0,2-CE

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit variant 100 R (tubes 0.5 ml) **REF** B5-100-R0,5-CE

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit variant 100 R (tubes 0.2 ml) **REF** B24-100-R0,2-CE

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit variant 100 R (tubes 0.5 ml) **REF** B24-100-R0,5-CE

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit variant 100 R, **REF** B5-100-R0,2-CE, **REF** B5-100-R0,5-CE includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-R *Neisseria gonorrhoeae* 1 ready-to-use single-dose test tubes (*under wax*)	colorless clear liquid	0.005	80 tubes of 0.5 or 0.2 ml
PCR-mix-1-R *Neisseria gonorrhoeae* 2 ready-to-use single-dose test tubes (*under wax*)	colorless clear liquid	0.005	30 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 *Neisseria gonorrhoeae*	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, K1-12-100-CE or DNA-sorb-B, K1-2-100-CE protocols).

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

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit variant 100 R, **REF** B24-100-R0,2-CE, **REF** B24-100-R0,5-CE includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-R *Neisseria gonorrhoeae* 1 ready-to-use single-dose test tubes (under wax)	colorless clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
PCR-mix-1-R *Neisseria gonorrhoeae* 2 ready-to-use single-dose test tubes (*under wax*)	colorless clear liquid	0,005	55 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 *Neisseria gonorrhoeae*	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, K1-12-100-CE or DNA-sorb-B, K1-2-100-CE protocols).

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

4. ADDITIONAL REQUIREMENTS

- DNA extraction 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 25-100 °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 instance, Axygen, USA).
- Refrigerator for 2–8 °C with 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 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 mucous 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.
- The laboratory process must be one directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. 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 is described in manufacturer's handbook [1]. It is recommended to read this handbook before starting work

AmpliSens® *Neisseria gonorrhoeae*-EPh PCR kit is intended to analyze DNA extracted by DNA extraction kits from the clinical material:

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

Storage of the samples in transport medium.

- at room temperature for 6 hours;
- at 2-8 ºC for 1 week;
- at minus 16 ºC for 1 month;
- at minus 68 ºC for a long time.

Urine sediment. Shake the dropper-bottle with urine specimen and transfer 1 ml of urine into 1.5 ml Eppendorf tube using the tip with aerosol filter. Spin the Eppendorf tube for 5 min at 10000 g. Remove the supernatant completely using vacuum aspirator. Add the required volume of transport medium to the pellet up to final volume of 0.2 ml. Vortex the tube thoroughly.

Storage of urine samples.

- at room temperature for 24 hours;
- at 2-8 ºC for 1 week;
- at minus 16 ºC for 2 months;
- at minus 68 ºC for a long time.

Storage of urine sediment and the secret of the prostate gland samples.

- at 2-8 ºC for 24 hours;
- at minus 16 ºC for 1 week;
- at minus 68 ºC for a long time.

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

Deliver the specimens in a container with an ice pack at 2-8 ºС for 5 days, or frozen specimens for 1 day.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extraction

It's recommended to use the following nucleic acid extraction kits:

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

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

8.2. Preparing PCR

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

8.2.1 Detection of DNA

Two pairs of primers are used for detection of *Neisseria gonorrhoeae* DNA:
- *Neisseria gonorrhoeae* 1 – for PCR amplification of cryptic plasmid DNA (395 bp);
- *Neisseria gonorrhoeae* 2 – for PCR amplification of aminoacetyltransferase gene (450 bp).

1. Collect the required quantity of the tubes prepared as describes above or tubes with **PCR-mix-1-R *Neisseria gonorrhoeae* 1** with wax for amplification of DNA of study and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of the wax layer of each tube ensuring that it does not fall under the wax.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Using tips with aerosol barrier add **10 µl** of **DNA samples**, obtained from clinical or control samples at the stage of DNA extraction
5. 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 *Neisseria gonorrhoeae*** to the tube for Positive Control of Amplification.

6. Prepare tubes with **PCR-mix-1-R *Neisseria gonorrhoeae* 2** as described above.

8.2.2 Amplification

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

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

Table 1

Programming thermocyclers at DNA amplification *Neisseria gonorrhoeae*

	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-2 (Biometra), MiniCycler, PTC-100 (MJ Research)**		
Step	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles	Tempe-rature	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.

When 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 lengths of specific amplified DNA fragments are:

- cryptic plasmid of *Neisseria gonorrhoeae* – 395 bp

- aminoacetyltransferase gene of *Neisseria gonorrheae* – 450 bp
- Internal Control complex – 660 bp

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

9.1. Results interpretation

The sample is considered to be positive for *Neisseria gonorrhoeae* DNA if positive result is detected for DNA of cryptic plasmid as well as for aminoacetyltransferase gene (with PCR-mix-1 *Neisseria gonorrhoeae* 1 and PCR-mix-1 *Neisseria gonorrhoeae* 2).

9.1.1. *Identifying of cryptic plasmid DNA (PCR-mix-1 Neisseria gonorrhoeae 1)*

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		395 bp	660 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 cryptic plasmid DNA of *Neisseria gonorrhoeae* if the band of 395 bp is present in the line regardless of the band of Internal Control. The band of IC could be absent in the samples with high concentration of *Neisseria gonorrhoeae* DNA.
- The sample is considered to be negative for cryptic plasmid DNA of *Neisseria gonorrhoeae* if the band of 660 bp is present and the band of 395 bp is absent.

Besides specific bands (395 bp and 660 bp) the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 nucleotide pairs.

9.1.2. *Identifying of aminoacetyltransferase gene DNA (PCR-mix-1 Neisseria gonorrhoeae 2)*

Table 3

Results for controls

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

- The sample is considered to be positive for aminoacetyltransferase gene DNA of *Neisseria gonorrhoeae* if the band of 450 bp is present in the line.
- The sample is considered to be negative for aminoacetyltransferase gene DNA of *Neisseria gonorrhoeae* if the band of 450 bp is absent.

Besides specific band (450 bp) the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 nucleotide pairs.

10. TROUBLESHOOTING

10.1. *Identifying of cryptic plasmid DNA (PCR-mix-1 Neisseria gonorrhoeae* 1*)*

Analysis results are not obtained as per the following examples:

- If the results of control samples do not correspond to the listed above (Table 2), then the tests should be repeated.
- If in the lane neither 395 nor 660 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of PCR.
- If nonspecific bands are presented in lines at different levels, 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 395 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 should be repeated and measures for detecting contamination source must be undertaken.

10.2. *Identifying of aminoacetyltransferase gene DNA (PCR-mix-1 Neisseria gonorrhoeae* 2*)*

Analysis results are not obtained as per the following examples:

- If the results of control samples do not correspond to the listed above (Table 3), then the tests should be repeated.
- If nonspecific bands are presented in lines at different levels, 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 450 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 should 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® *Neisseria gonorrhoeae*-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® *Neisseria gonorrhoeae*-EPh** PCR kit are to be stored at 2-8 ºC when not in use. All components of the PCR kit are to be stable until labeled expiration date. 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® *Neisseria gonorrhoeae*-EPh** PCR kit is no less than $5x10^3$ genome equivalents per1 ml of sample (GE/ml).

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

13.2. Specificity

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

14. REFERENCES

1. Manual “Sampling, transportation and 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® *Neisseria gonorrhoeae*-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		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”