



For *in Vitro* Diagnostic Use

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP

PCR kit

Instruction Manual



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1. INTENDED USE.

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Neisseria gonorrhoeae* DNA in the clinical materials (cervical or urethral scrapes (swabs); fauces, conjunctival, anorectal swabs; urine sediment; secret of the prostate gland; synovial fluid) by means of end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Neisseria gonorrhoeae detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special primers. In Fluorescent End-Point PCR, the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening of the reaction tubes after the PCR run. **AmpliSens® *Neisseria gonorrhoeae*-FEP** PCR kit is a qualitative test and contains the Internal Control (IC) which must be used in the isolation procedure in order to control the isolation process of each individual specimen and to identify possible reaction inhibition. **AmpliSens® *Neisseria gonorrhoeae*-screen-FEP** 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 °C, which greatly diminish frequency of nonspecifically primed reactions.

3. CONTENT.

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit is produced in 2 forms:

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit (tubes of 0.2 ml) **REF** B51-100-R0,2-FEP-CE;

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit (tubes of 0.5 ml) **REF** B51-100-R0,5-FEP-CE;

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT <i>Neisseria gonorrhoeae</i>-screen (under wax)	colorless, clear liquid	0.008	110 tubes of 0.2 or 0.5 ml
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
PCR-mix-Background	colorless, clear liquid	0.5	1 tube
Mineral oil for PCR	colorless, viscous liquid	4.0	1 vial
Positive Control DNA <i>Neisseria gonorrhoeae</i> (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 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** K1-12-100-CE, DNA-sorb-B **REF** K1-2-100-CE protocols).

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters up to 200 µl.
- Tube racks.
- 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, USA), Uno-2 (Biometra, Germany), Terzik (DNA-Technology, Russia);
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity.
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature below minus 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 use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens 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 sample or reagent contact with the skin, eyes and mucose membranes. If any of 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 unidirectional manner, beginning in the Extraction Area and moving 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.



Sampling of biological material for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting of the work.

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit is intended to analyze DNA extracted with DNA isolation kits from

- *cervical, urethral scrapes (swabs);*
- *fauces, conjunctival, anorectal swabs;*
- *urine sediment;*

— *secret of the prostate gland;*

— *synovial fluid.*

7. PROTOCOL.

7.1. DNA Isolation

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

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

7.2. Preparing the PCR.

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



Tube type depends on the Instrument to be used:

- 0.2 ml tubes should be used with "Gradient Palm Cycler", "GeneAmp PCR System 2700", "Uno-2", "ALA-1/4".
- 0.5 ml tubes should be used with "Terzik", ALA-1/4.

7.2.1 Preparing tubes for PCR.

1. Prepare the required quantity of tubes with **PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-screen** with wax for amplification of DNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-screen.
3. Add above **1 drop of mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-screen** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of the wax layer of each tube, ensuring that it does not fall under the wax and mix with PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-screen. Add above **1 drop of mineral oil for PCR**.



Background samples, that have been thermocycled once, can be used for further runs without thermo cycling. Multiple use of Background samples is permitted only if the PCR kit of the same lot is applied. Store the Background tubes from 2 to 25 °C for up to 1 week. Keep away from light.

5. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction.



The tubes with PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-screen that are not used at the moment should be kept away from light.

6. Carry out 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 DNA *Neisseria gonorrhoeae*** to the tube labeled C+ (Positive Control of Amplification).

7.2.2 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 the button to continue.

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

Table 1

Programming thermocyclers at DNA amplification of *Neisseria gonorrhoeae* (65-60-45)

Step	Thermocyclers with active temperature adjustment:									Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (ABI)			"Gradient Palm Cycler" (Corbett Research)			"Uno-2" (Biometra)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		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	95 °C	5 min	1
2	95 °C	2 sec	35	95 °C	20 sec	20	95 °C	2 sec	24	95 °C	25 sec	20
	65 °C	5 sec		65 °C	25 sec		65 °C	10 sec		65 °C	40 sec	
	72 °C	5 sec		72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
3	95 °C	2 sec	9	95 °C	20 sec	24	95 °C	2 sec	19	95 °C	25 sec	24
	60 °C	10 sec		60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
	72 °C	5 sec		72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
4	95 °C	2 sec	1	95 °C	20 sec	1	95 °C	2 sec	1	95 °C	25 sec	1
	60 °C	10 sec		60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
5	10 °C	storage		10 °C	storage		10 °C	storage		10 °C	storage	



Detection can be performed within 1 day from the end of the amplification in case the tubes have been stored at below 28 °C and protected from light.

8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector (Biosan, Latvia).



Please read Aladin Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Appendix 1.

Results interpretation

1. When the analysis is complete the results are automatically shown in the table in the manner of following indications:

pos – positive result;

neg – negative result;

eq – equivocal result (signal is in grey zone);

nd – invalid result (specific signal and IC signal are absent in the sample).

2. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2).

Table 2 Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (samples)	HEX channel (IC)	
C-	DNA isolation	<i>Neisseria gonorrhoeae</i> – neg	+	OK
NCA	Amplification	<i>Neisseria gonorrhoeae</i> – nd	-	OK
C+	Amplification	<i>Neisseria gonorrhoeae</i> – pos	-	OK

9. TROUBLESHOOTING.

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

- No signal with positive control of PCR (C+) can indicate incorrect programming of the temperature profile of

the thermocycler or incorrect configuration of the PCR reaction or storage conditions for kit components did not comply with manufacturer instruction or reagents kit had expired. It is necessary to check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again.

- If no signal was detected in both channels, for detection of pathogen DNA and Internal Control, the sample should be examined repeatedly (PCR and detection). Similar procedures should be applied for the samples with equivocal result, that is, specific signal exceeds the background not enough to consider the sample as positive. If the same result is registered in the second run, the analysis should be repeated starting from the stage of DNA extraction.
- Positive signal in negative controls (C- or NCA) indicates the reagents or samples contamination. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens[®] *Neisseria gonorrhoeae*-screen-FEP** PCR kit are to be stored between 2°C and 8°C, when not in use. All components must be stable until the expiry date stated on the label.



PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae* should be kept away from light.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens[®] *Neisseria gonorrhoeae*-screen-FEP** PCR kit is no less than 1x10³ genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of **AmpliSens[®] *Neisseria gonorrhoeae*-screen-FEP** PCR kit are guaranteed only when additional reagents kits, "DNA-sorb-AM" or "DNA-sorb-B" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), are used.

11.2. Specificity.

Specificity of **AmpliSens[®] *Neisseria gonorrhoeae*-screen-FEP** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens[®] *Neisseria gonorrhoeae*-screen-FEP** PCR kit was confirmed in laboratory clinical trials.

















12. REFERENCES.

- 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 Quality Management System, each lot of **AmpliSens® Neisseria gonorrhoeae-screen-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control