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For Professional Use Only

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

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

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 material (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) using end-point hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

Neisseria gonorrhoeae DNA detection by the polymerase chain reaction (PCR) is based on the amplification of a pathogen genome specific region using special *Neisseria gonorrhoeae* primers. In Fluorescent End-Point PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in the reaction mixture after the PCR. It allows the detection of the accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® ***Neisseria gonorrhoeae*****-screen-FEP** PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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® ***Neisseria gonorrhoeae*****-screen-FEP** PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 °C.

3. CONTENT

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

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

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

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

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *Neisseria gonorrhoeae*-screen (ready-to-use single-dose test tubes (*under wax)*)	colorless clear liquid	0.01	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
Mineral oil for PCR*	colorless viscous liquid	4.0	1 dropper bottle
PCR-mix-Background-red**	red clear liquid	0.6	1 tube
Positive Control complex (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

* is used for thermocyclers without constant-temperature lid.

** is used to analyze DNA samples extracted with **DNA-sorb-AM** and **DNA-sorb-B** extraction kits.

*** must be used in the extraction procedure as Negative Control of extraction.

****add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **DNA-sorb-AM** **REF** K1-12-100-CE protocol).

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

4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), MaxyGene (Axygen, USA), or equivalent).

- Fluorometer (ALA-1/4 (Biosan, Latvia) or equivalent instrument).
- Refrigerator for 2–8 °C.
- Deep-freezer for the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge them briefly.
- Use disposable protective gloves, laboratory coats, and protect eyes while samples and reagents handling. 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 samples and unused reagents in compliance with the local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where 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 [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP PCR kit is intended for analysis of DNA extracted with the use of DNA extraction kits from the clinical material (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; urine samples, or the prostate gland secretion).

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1 DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- **DNA-sorb-AM**, **REF** K1-11-100-CE.
- For other nucleic acid extraction kits see Guidelines [2].

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC).**

In the extraction procedure it is necessary to carry out the control reactions as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labeled C– (Negative Control of Extraction).

Extract DNA according to the manufacturer's instructions.

8.2 Preparing PCR

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

8.1.1 Preparing tubes for PCR

1. Prepare the required number of tubes with **PCR-mix-1-FL *Neisseria gonorrhoeae*-screen** for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of the wax layer into each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *Neisseria gonorrhoeae*-screen.**
3. Add above **1 drop** of **mineral oil for PCR** if a thermocycler without constant-temperature lid is used.

4. Prepare one **Background** sample. To do this, mark one **PCR-mix-1-FL *Neisseria gonorrhoeae*-screen** tube as **Background** and add **20 µl** of **PCR-mix-Background-red** above the wax layer surface ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *Neisseria gonorrhoeae*-screen**. Add above **1** drop of **mineral oil for PCR** (if a thermocycler without a constant-temperature lid is used).

PCR-mix-Background-red is used if DNA was extracted using **DNA-sorb-AM** (**REF** K1-12-100-CE) or **DNA-sorb-B** (**REF** K1-2-100-CE). If any other nucleic acid extraction kit (recommended by FBIS CRIE) was used, follow the instructions provided by the manufacturer.

5. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
6. Carry out the 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 complex (C+)** to the tube labeled **C+** (Positive Control of Amplification).

C– – Add **10 µl** of **the sample extracted from Negative Control (C–) reagent** to the tube labeled **C–** (Negative Control of Extraction).

8.1.1 **Amplification**

1. Run the following program in the thermocycler (see Table 1).
2. When the temperature reaches 95 °C (pause mode), insert tubes into the thermocycler cells and press the button to continue.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the thermocycler.

Table 1

AmpliSens-1-FEP amplification program

	GeneAmp PCR System 2700			**Gradient Palm Cycler, MaxyGene**		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
0	**95**	pause		**95**	pause	
1	**95**	5 min	1	**95**	5 min	1
2	**95**	20 s	20	**95**	2 s	24
	65	25 s		**65**	10 s	
	72	30 s		**72**	10 s	
3	**95**	20 s	24	**95**	2 s	20
	60	30 s		**60**	15 s	
	72	30 s		**72**	10 s	
4	**95**	20 s	1	**95**	2 s	1
	60	30 s		**60**	15 s	
5	**10**	storage		**10**	storage	

Amplification programs for some other models of thermocyclers are specified in Guidelines [2].

3 Proceed to fluorescence detection after the amplification program is completed.

9. DATA ANALYSIS

Detection is conducted using a fluorescence detector.

Please read ALA-1/4 Operating Manual before using this kit.

The detection is performed by means of a fluorescence detector by measuring the fluorescence signal intensity in two channels:

– The channel for the FAM fluorophore (FAM channel or analogous, depending on the detector model) is intended for the detection of the signal of the *Neisseria gonorrhoeae* DNA amplification product.
– The channel for the JOE fluorophore (HEX channel or analogous, depending on the detector model) is intended for the detection of the signal of the IC DNA amplification product.

Before the detection run, the required settings of the detector software should be adjusted according to the *Important Product Information Bulletin* enclosed to the PCR kit and Guidelines [2].

The obtained results are interpreted on the basis of the level of fluorescence signal in the corresponding channels relatively to the background for the clinical and control samples. Interpretation is performed automatically by the software of the instrument used.

The principle of interpretation is the following:

- *Neisseria gonorrhoeae* DNA is **detected** in a sample if the signal determined in the channel for the FAM fluorophore is greater than the specified threshold value of the positive result.
- *Neisseria gonorrhoeae* DNA is **not detected** in a sample if the signal determined in the channel for the FAM fluorophore is less than the specified threshold value of the negative result, whereas the signal determined in the channel for the JOE fluorophore is greater than the specified threshold value.
- The result is **invalid** in a sample if the signal determined in the channel for the FAM fluorophore is less than the specified threshold value of the negative result, whereas the signal determined in the channel for the JOE fluorophore is less than the specified threshold value. In such cases, the PCR analysis of this sample should be repeated.
- The result is **equivocal** if the signal of a sample determined in the channel for the FAM fluorophore is greater than the specified threshold value of the negative result but less than the threshold value of the positive result (the signal is between thresholds). In such cases, the PCR analysis of this sample should be repeated.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 2).

Table 2

Results for controls

Control	Stage for control	Fluorescent signal in the channel for the fluorophore	
		FAM	JOE
C–	DNA extraction	**<** threshold value of negative result	**>** threshold value
NCA	PCR	**<** threshold value of negative result	**<** threshold value
C+	PCR	**>** threshold value of positive result	**>** threshold value

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If the signal determined for the Positive Control of amplification (C+) in the FAM channel is less than the threshold value of positive result, the amplification and detection should be repeated for all samples in which *Neisseria gonorrhoeae* DNA was not detected.
2. If the signal determined for the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA) in the FAM channel is greater than the threshold value of positive result, the PCR analysis should be repeated starting from the DNA extraction stage for all samples in which *Neisseria gonorrhoeae* DNA was detected.

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

11. TRANSPORTATION

AmpliSens® *Neisseria gonorrhoeae*-screen-FEP 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*-screen-FEP** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Neisseria gonorrhoeae*-screen-FEP** PCR kit are stable until the expiration date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FL *Neisseria gonorrhoeae*-screen is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Neisseria gonorrhoeae*-screen-FEP** PCR kit is specified in the table below.

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml [1]
Urogenital swabs	Transport Medium for Swabs (REF 956-CE, REF 987-CE) or Transport Medium with Mucolytic Agent (REF 952-CE, REF 953-CE)	DNA-sorb-AM	5×10^2
Urine (pretreatment is required)	—	DNA-sorb-AM	1×10^3

13.2. Specificity

The analytical specificity of **AmpliSens® *Neisseria gonorrhoeae*-screen-FEP** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Nonspecific reactions were absent while testing human DNA samples and a DNA panel of following microorganisms: *Neisseria flava, N.subflava, N.sicca, N.mucosa, N.lactamica,* and *N.meningitides; Gardnerella vaginalis; Lactobacillus* spp.; *Escherichia coli; Staphylococcus aureus; Streptococcus pyogenes and S. agalactia; Candida albicans; Mycoplasma hominis* and *M.genitalium; Ureaplasma urealyticum* and *U.parvum; Chlamydia trachomatis; Treponema pallidum; Trichomonas vaginalis; Toxoplasma gondii; HSV* types 1 and 2; *CMV;* and *HPV.*

The clinical specificity of **AmpliSens® *Neisseria gonorrhoeae*-screen-FEP** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines “End-Point PCR Detection of STIs and Other Reproductive Tract Infections.”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

[1] Genome equivalents (GE) of the microorganism per 1 ml of the sample placed into the specified transport medium.

15. QUALITY CONTROL

In compliance with the Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *Neisseria gonorrhoeae*-screen-FEP** PCR kit has been 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		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
02.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
23.11.15 ME	Text	Corrections according to the template
	8.1. DNA extraction	Information about controls of extraction was added
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	