



For *in Vitro* Diagnostic Use

AmpliSens® *Gardnerella vaginalis*-FEP

PCR kit

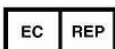
Instruction Manual

AmpliSens®

TABLE OF CONTENTS

1. INTENDED USE.....	1
2. PRINCIPLE OF PCR DETECTION.....	2
3. CONTENT.....	2
4. ADDITIONAL REQUIREMENTS.....	2
5. GENERAL PRECAUTIONS.....	3
6. SAMPLING AND HANDLING.....	3
7. PROTOCOL.....	3
8. DATA ANALYSIS.....	4
9. TROUBLESHOOTING.....	5
10. STABILITY AND STORAGE.....	5
11. SPECIFICATIONS.....	5
12. REFERENCES.....	5
13. QUALITY CONTROL.....	6
14. EXPLANATION OF SYMBOLS.....	6

AmpliSens®



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1. INTENDED USE.

AmpliSens® *Gardnerella vaginalis*-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Gardnerella vaginalis* DNA in the clinical materials (cervical, urethral scrapes (swabs), urine sediment, secrete of the prostate gland) by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Gardnerella vaginalis detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Gardnerella vaginalis* primers. In Fluorescent End-Point PCR, the amplified product is detected by 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 the reaction tubes after the PCR run. AmpliSens® *Gardnerella vaginalis*-FEP PCR kit is a qualitative test, which contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. AmpliSens® *Gardnerella vaginalis*-FEP 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 (or chemically modified polymerase (TaqF)). The wax melting and reaction mix component occurs only at 95°C. Chemically modified polymerase (TaqF) activates by heating at 95°C for 15 min.

3. CONTENT.

AmpliSens® *Gardnerella vaginalis*-FEP PCR kit is produced in 2 forms:
 AmpliSens® *Gardnerella vaginalis*-FEP PCR kit variant FEP (tubes 0.5 ml), **REF** B4-100-R0,5-FEP-CE.
 AmpliSens® *Gardnerella vaginalis*-FEP PCR kit variant FEP (tubes 0.2 ml), **REF** B4-100-R0,2-FEP-CE.

AmpliSens® *Gardnerella vaginalis*-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>Gardnerella vaginalis</i> ready-to-use single-dose test tubes (<i>under wax</i>)	colorless, clear liquid	0.008	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
Mineral oil for PCR	colorless, viscous liquid	4.0	1 dropper bottle
PCR-mix-Background	colorless, clear liquid	0.5	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**	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 protocol).

AmpliSens® *Gardnerella vaginalis*-FEP PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.


- DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barriers (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), GeneAmp PCR System 2700 or GeneAmp PCR System 2400 (ABI), Uno-2 (Biomtra), MiniCycler, PTC-100 (MJ Research);
- Fluorometer ALA-1/4 ("Biosan", Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for temperature between 2 and 8 °C
- Deep-freezer with temperature not more than minus16 °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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.
Amplisens® Gardnerella vaginalis-FEP PCR kit is intended for analysis of DNA extracted by using DNA isolation kits from cervical or urethral scrapes (swabs), urine sediment (use the first part of the stream), or secrete of the prostate gland.

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.

 Carry out the DNA isolation according to the manufacturer's instructions.

7.2. Preparing the PCR.

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

7.2.1 Preparing tubes for PCR.

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT Gardnerella vaginalis** and 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 Gardnerella vaginalis**.
3. Add above **1** drop of **mineral oil for PCR** (about **25 µl**) – not necessary if the cycler has heated lid.
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT Gardnerella vaginalis** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of wax layer of each tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT Gardnerella vaginalis**. Add above **1** drop of **mineral oil for PCR** – not necessary if the cycler has heated lid..



Background samples, that have been thermocycled once, can be used for further runs without further thermo cycling. Multiple applications of Background samples are permitted only if the PCR kit of the same lot is applied. Store the Background tubes at 2-25 °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 DNA extraction stage.



The tubes with PCR-mix-1-FEP/FRT *Gardnerella vaginalis* that are not used at the moment should be stored away from light.

6. Carry 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** 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 reaches 95°C (pause regimen), insert tubes to the cells of amplifier and press the button to continue.

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

Table 1

Programming thermocyclers at DNA amplification of *Gardnerella vaginalis* (65-60-40)

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" (Biomtra)		
	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	

8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read ALA 1/4 Operating Manual before use of this kit.

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



Detection can be conducted within 1 day from the end of the amplification only if the tubes with amplified product have been stored at 28°C or below in a light-free area.

Results interpretation

1. When the analysis is complete the results are automatically shown in the table as follows:

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	Controlled stage	Results		Interpretation
		FAM channel (samples)	HEX channel (IC)	
C-	DNA isolation	<i>Gardnerella vaginalis</i> - neg	+	OK
NCA	Amplification	<i>Gardnerella vaginalis</i> - nd	-	OK
C+	Amplification	<i>Gardnerella vaginalis</i> – pos	-	OK

9. TROUBLESHOOTING.

- If analysis results are not obtained as per the following examples:

No positive signal with positive control of PCR (C+) can indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components not complying with manufacturer instruction, or reagent kit has expired. Check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.

- If no signal was detected in either the channel for detection of pathogen DNA or the channel for detection of Internal Control, the sample should be examined again (PCR and detection). The same procedures should be applied to the samples with questionable results, that is, the specific signal does not exceed background enough to consider the sample as positive. If questionable results obtained in the second run, the analysis should be repeated starting from the stage of DNA extraction.

- Positive signal in negative controls (C-, NCA) indicates the reagent or sample contamination. In such case results of analysis must be considered as inconclusive. The analyses must be repeated and measures taken detect and eliminate the contamination source.

10. STABILITY AND STORAGE.

All components of the **AmpliSens® Gardnerella vaginalis-FEP** PCR kit are to be stored at the temperature between 2°C and 8°C, when not in use. All components of the **AmpliSens® Gardnerella vaginalis-FEP** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Gardnerella vaginalis-FEP** PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® Gardnerella vaginalis-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® Gardnerella vaginalis-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® Gardnerella vaginalis-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® Gardnerella vaginalis-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