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For Professional Use Only

AmpliSens® *Gardnerella vaginalis*-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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 clinical materials (urogenital swabs) by 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

Gardnerella vaginalis DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Gardnerella vaginalis* primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected by using 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 a reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Gardnerella vaginalis*-FEP** PCR kit is a qualitative test that contains 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® *Gardnerella vaginalis*-FEP** PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C.

3. CONTENT

AmpliSens® *Gardnerella vaginalis*-FEP PCR kit is produced in 2 forms:

AmpliSens® *Gardnerella vaginalis*-FEP PCR kit variant FEP (0.5-ml tubes),

REF B7-100-R0,5-FEP-CE.

AmpliSens® *Gardnerella vaginalis*-FEP PCR kit variant FEP (0.2-ml tubes),

REF B7-100-R0,2-FEP-CE.

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

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *Gardnerella vaginalis* (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 (DNA-sorb-AM **REF** K1-12-100-CE).

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

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- 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 a 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) MaxyGene (Axygen, USA) or equivalent).
- Fluorometer (for example, ALA-1/4 (Biosan, Latvia) or equivalent).
- Personal computer.

- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml) (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, 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 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 another 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 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 are 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 extraction kits from urogenital swabs.

7. WORKING CONDITIONS

AmpliSens® *Gardnerella vaginalis*-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.

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**.

8.2.1 **Preparing tubes for PCR**

1. Prepare the required number of tubes with **PCR-mix-1-FL *Gardnerella vaginalis*** 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 of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *Gardnerella vaginalis*.**
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**) if a thermocycler without constant-temperature lid is used.
4. Prepare one **Background** sample. To do this, mark one **PCR-mix-1-FL *Gardnerella vaginalis*** 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 *Gardnerella vaginalis***. 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 from clinical or control samples at the DNA extraction stage using tips with aerosol barrier.
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** to the tube labeled C+ (Positive Control of Amplification).

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

8.2.2 **Amplification**

Run the program **AmpliSens-1-FEP amplification program** on the thermocycler (see table 1).

When the temperature reaches 95 ºC (pause mode), insert tubes into cells of thermocycler and press the button to continue.

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

Table 1

AmpliSens-1-FEP amplification program

	GeneAmp PCR System 2700 (Applied Biosystems, USA)			**Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygen, USA)**		
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	

After the amplification program run finishes, analyze and record results.

Amplification programs for different thermocycler models are described in **Guidelines "End-Point PCR Detection of STIs and Other Reproductive Tract Infections"** [2].

9. DATA ANALYSIS

Detection is conducted using a fluorescence detector.

Please read the fluorescence detector Operating Manual before using this kit.

Detection can be conducted within 1 day after completion of amplification only if the tubes with the amplified product have been stored at 2–8 °C in a light-free area.

Program the detector according to the manufacturer's manual and Guidelines.

The fluorescent signal intensity is detected in two channels:

– the signal from the ***Gardnerella vaginalis*** DNA amplification product is detected in the FAM channel (or analogous, depending on the detector model);

– the signal from the **IC** amplification product is detected in the HEX channel (or analogous, depending on the detector model).

Principle of interpretation:

1. *Gardnerella vaginalis* DNA is **detected** in a sample if its signal in the FAM channel is

greater than the defined threshold value of the positive result.

2. *Gardnerella vaginalis* DNA is **not detected** in a sample if the signal in the FAM channel is less than the defined threshold value of the negative result whereas the signal in the HEX channel is greater than the defined threshold value.
3. The result is **invalid** in a sample if the signal in the FAM channel is less than the defined threshold value of the negative result whereas the signal in the HEX channel is less than the defined threshold value.

If the result is invalid or equivocal, PCR should be repeated once again.

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

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (samples)	**HEX channel** (IC)	
C–	DNA extraction	**<** threshold of negative result	**>** threshold	"-" or OK
NCA	Amplification	**<** threshold of negative result	**<** threshold	"nd"
C+	Amplification	**>** threshold of positive result	**>** threshold	"+" or OK

10. TROUBLESHOOTING

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

1. No positive signal in the positive control of amplification (C+) may indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of PCR, noncompliance of the storage conditions for kit components with the manufacturer's instruction, or the expiration of the reagent kit. Check programming of the thermocycler (see 8.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR once again for all samples.
2. If no signal was detected either in the channel for detection of the pathogen DNA or in the channel for detection of the Internal Control, the sample should be examined once again (PCR and detection). The same applies to the samples with equivocal results, because the fact that the specific signal does not exceed the threshold value is not sufficient to consider a sample as positive. If equivocal results are obtained in the second run, the analysis should be repeated starting from the DNA extraction stage.
3. Positive signal in negative controls (C– and NCA) indicates reagent or sample contamination. In this case, the results of analysis must be considered as invalid. The analyses must be repeated and measures for detecting and eliminating the contamination source must be taken.

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

11. TRANSPORTATION

AmpliSens® *Gardnerella vaginalis*-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® *Gardnerella vaginalis*-FEP** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Gardnerella vaginalis*-FEP** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FL *Gardnerella vaginalis* should be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Gardnerella vaginalis*-FEP** PCR kit is as follows:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml [1]
Urogenital swabs[2]	DNA-sorb-AM	$2x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® *Gardnerella vaginalis*-FEP** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: *Lactobacillus spp.; Escherichia coli; Staphylococcus* spp.; *Streptococcus* spp.; *Mycoplasma hominis; Ureaplasma urealyticum; Ureaplasma parvum; Candida albicans; Neisseria spp.; Neisseria gonorrhoeae; Mycoplasma genitalium; Trichomonas vaginalis; Treponema pallidum; Toxoplasma gondii; HSV* types 1 and 2, *CMV,* and *HPV*. The clinical specificity of **AmpliSens® *Gardnerella vaginalis*-FEP** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR

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

[2] Urogenital swabs are to be placed into the Transport Medium for Swabs (**REF** 956-CE, 987-CE) or Transport Medium with Mucolytic (**REF** 952-CE, 953-CE).

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, 2008.

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" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for 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.

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
19.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”