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For Professional Use Only

AmpliSens® *Gardnerella vaginalis* / *Lactobacillus* spp.-titre-FRT PCR kit

Instruction Manual

AmpliSens®

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Science “Central Research
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1. INTENDED USE

AmpliSens® *Gardnerella vaginalis* / *Lactobacillus* spp.-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantitation of *Gardnerella vaginalis* and *Lactobacillus* species DNA in the clinical materials by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Gardnerella vaginalis and *Lactobacillus* species detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Gardnerella vaginalis* and *Lactobacillus* species primers. In real-time 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. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Gardnerella vaginalis* / *Lactobacillus* spp.-titre-FRT** 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 chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95ºC for 15 min.

According to the analysis results (if all procedures are strictly followed) the vaginal microcenosis can be estimated and the bacterial vaginosis can be diagnosed with high accuracy. The bacterial vaginosis is a disease associated with reduction of normal bacterial flora of vagina and substitution with opportunistic one.

Bacterial vaginosis (BV) is an infectious noninflammatory syndrome caused by reduction or complete absence of lactobacilli that suppress pathogenic bacteria flora of vagina and consequently overgrowth of opportunistic microorganisms, first of all *Gardnerella vaginalis*.

Normally, vaginal flora consists of *Lactobacillus* species (95-98%) and its concentration varies from 10^6 to 10^{10}CFU/ml. The main vaginal species produce H_2O_2 that prevent multiplying of opportunistic bacteria (*Gardnerella vaginalis*, *Mobiluncus* spp., etc.). Normally, their concentration does not exceed 10^3-10^5 CFU/ml. In addition to it lactobacilli acidulate pH of vaginal discharge (normal pH does not exceed 4.5) by metabolizing of

glycogen until lactic acid is formed, that provide inhibition of anaerobic microorganisms growth.

Regardless of reasons that caused BV, reduction of *Lactobacillus* growth occurs. It leads to opportunistic microorganisms boost, first of all *Gardnerella vaginalis*, and its waste products create favorable conditions for growth of other opportunistic; microorganisms. It was proved that *Gardnerella vaginalis* was found in 100% in case of BV so it was a main marker of BV. Until recently, *Gardnerella vaginalis* was considered to be a main causative agent of BV. On the other hand, normally *Gardnerella vaginalis* is found at a high rate, 50-60%. Therefore, the detection of *G.vaginalis* even by bacteriological technique is a low specific marker. Specificity can be increased by determination of quantitative characteristics of the marker, that is, to value the concentration of *Gardnerella vaginalis*.

However, in some cases, concentration of *Gardnerella vaginalis* in BV absence along with normal concentration of *Lactobacilli*, depending on a day of menstrual cycle, can reach 10^7-10^8 CFU/ml. The most accurate marker of BV is a logarithmic relation of *Lactobacillus* spp. and *Gardnerella vaginalis* concentrations.

The method of *Lactobacillus* spp. and *Gardnerella vaginalis* DNA assay in clinical material is based on:

a) Total DNA isolation from cell suspension.

Clinical material is to be placed into Transport media with mucolytic agent **REF** 952 manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

b) Simultaneous real-time amplification (multiplex-PCR) of *Lactobacillus* spp. and *Gardnerella vaginalis* DNA specific regions. *Gardnerella vaginalis* DNA is registered on FAM channel and *Lactobacillus* spp. DNA is registered on JOE (HEX) channel.

Quantitative calibrators are used for *Gardnerella vaginalis* and *Lactobacillus* spp. DNA copies assay in standard volume of clinical sample

Calculation of concentrations of *Gardnerella vaginalis* and *Lactobacillus* spp. DNA per 1 ml of a clinical material (posterior fornix of vagina discharge):

$$K_{DNA\ Gv/ml} = K_{DNA\ Gv} * \text{coefficient}$$

$$K_{DNA\ Lsp/ml} = K_{DNA\ Lsp} * \text{coefficient}$$

$K_{DNA\ Gv}$ = copies quantity of *Gardnerella vaginalis* DNA per reaction,

$K_{DNA\ Lsp}$ = copies of *Lactobacillus* spp. DNA per reaction,

Coefficient=100 takes into account the volume of DNA in the reaction tube from the volume of the clinical material and the quantity of copies of the amplified gene in the genome of the microorganism.

Calculation of relation coefficient of *Lactobacillus* spp. and *Gardnerella vaginalis* concentrations:

$$KC_{Lsp\text{-}Gv} = lg[K_{DNA\ Lsp/ml}] - lg[K_{DNA\ Gv/ml}]$$

KC < -1.0 – high possibility of BV

KC > 2.0 – low possibility of BV

3. CONTENT

AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT PCR kit is produced in 1 form:

AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT PCR kit variant titre-FRT-100 F **REF** R-B7-FT(RG,iQ,Mx)-CE;

AmpliSens ® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT PCR kit, variant titre-FRT-100 F includes:

Reagent		***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp.		colorless clear liquid	0.8	1 tube
PCR-buffer-FRT		colorless clear liquid	0.9	1 tube
Polymerase (TaqF)		colorless clear liquid	0.06	1 tube
DNA-buffer		colorless clear liquid	0.5	1 tube
DNA calibrators PC *Gardnerella vaginalis / Lactobacillus* spp.	**GL1**	colorless clear liquid	0.06	1 tube
	GL2	colorless clear liquid	0.06	1 tube
	GL3	colorless clear liquid	0.06	1 tube
Positive Control DNA *Gardnerella vaginalis / Lactobacillus* spp.-1	**BV-**	colorless clear liquid	0.05	1 tube
Positive Control DNA *Gardnerella vaginalis / Lactobacillus* spp.-2	**BV+**	colorless clear liquid	0.05	1 tube

AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).

- Sterile pipette filter tips (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia); iQ5 or iQ iCycler (BioRad, USA); Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene microtubes for PCR with 0.2 ml capacity (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 filter tips 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 / Lactobacillus* spp.-titre-FRT PCR kit is intended to analyze DNA extracted with DNA isolation kits from:

- *Discharge of posterior fornix of vagina*

6.1. *Discharge of posterior fornix of vagina* (0.05± 0.01 ml) should be obtained by universal probe or "Copan" (Italy) applicator and placed into 2 ml tube containing TSM transport medium (0.5 ml). Rinse the effective part of the probe in transport medium and press by tube walls.

If the sample volume is sufficient the transport medium should become opaque and change its color from pink to yellow (pH of vagina discharge is acidic). If the color has not changed we recommend taking additional portion of the sample with new probe. Color of the medium is not affected if pH of the sample more than 4.5.

The sample put into transport medium with mucolytic agent can be stored and transported in firmly sealed tubes:

- up to 28 days at 18–25 °C
- up to 3 months at 2–8 °C
- for long-term storage the samples are to be frozen at minus 20 °C or lower

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

7. WORKING CONDITIONS

AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

Different manufacturers offer DNA extraction kits. We recommend following nucleic acid

extraction kits:

- ”DNA-sorb-AM”, **REF** K1-12-100-CE.

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

Add 100 µl of TSM transport medium to the tube labeled Negative Control of Extraction during extraction procedure.

To the Positive Control of extraction tubes ((BV-) and (BV+)) transfer 90 µl of TSM transport medium (per each) and 10 µl of PC DNA *Gardnerella vaginalis / Lactobacillus* spp.-1 or PC DNA *Gardnerella vaginalis / Lactobacillus* spp.-2 (respectively).

8.2. Preparing the PCR

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

8.2.1 Preparing tubes for PCR

1. **Preparing of** PCR-buffer-FRT and Polymerase (TaqF) mix. Into the tube with PCR-buffer-FRT (0.9 ml) add all content of the tube of Polymerase (TaqF) (0.06 ml) and vortex carefully; avoid foaming. Label the tube indicating the date of preparation. Use disposable filter tips only.

Prepared mix is intended for 120 samples.
Store at 2 – 8 ºC during 3 months.

2. Prepare the required number of the tubes for amplification of DNA from clinical and control samples.
3. Add reagents into the tubes (see Table 1).

Table 1

Adding of reagents

Method 1	Method 2
1. Add **7 µl of PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp**. into each tube 2. Add above **8 µl of prepared mix of PCR-buffer-FRT and Polymerase (TaqF)**	1. Prepare reaction mix for required number of reactions, calculating per each reaction: – **7 µl of PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp.** – **8 µl of prepared mix of PCR-buffer-FRT and Polymerase (TaqF).** While calculating, take into account four controls (Negative Control and three Calibrators) and one extra reaction (see Table 2). 2. Add **15 µl** of prepared mix into the tubes.

Table 2.

Scheme of reaction mix preparation

Samples to be examined:	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PCR-mix-1-FRT *Gardnerella vaginalis* / *Lactobacillus* spp., µl	56	63	70	77	84	91	98	105	112	119	126	133	140	147	154
Mix of PCR-buffer-FRT and Polymerase (TaqF), µl	64	72	80	88	96	104	112	120	128	136	144	152	160	168	176
Samples to be examined:	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
PCR-mix-1-FRT *Gardnerella vaginalis* / *Lactobacillus* spp., µl	161	168	175	182	189	196	203	210	217	224	231	238	245	252	259
Mix of PCR-buffer-FRT and Polymerase (TaqF), µl	184	192	200	208	216	224	232	240	248	256	264	272	280	288	296

4. Using filter tips add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared tubes.

Avoid transferring of sorbent into reaction mix when adding DNA.

5. Carry out the control and calibration amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification) instead of the DNA-sample

Calibrators PC (GL1, GL2, GL3) - Into three tubes add 10 µl of each DNA-calibrator (GL1, GL2, GL3)

8.2.2. Amplification

8.2.2.1. RG

1. Program Rotor-Gene ™ according to manufacturer's manual and the Guidelines (see Tables 3-4).
2. Create a temperature profile on Rotor-Gene ™ instrument as follows:

Table 3

Rotor-Gene 3000/6000 amplification program

Step	***Temperature °C***	***Time***	***Fluorescence detection***	***Repeats***
Hold	95	15 min	–	1
Cycling	95	10 sec	–	45
	60	40 sec	FAM/Green, JOE/Yellow	

Universal program**, AmpliSens-1 RG**, can be used as well (see table 4). The program allows conducting any combination of tests (for example, for detection of DNA of sexually transmitted infections) in a single run.
Analytical performances of the reagents kits remain the same when applying universal amplification program.

Table 4

AmpliSens-1 RG amplification program

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Repeats*
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 sec		

Note. ROX/Orange and Cy5/Red channels are activated when necessary if multiplex format tests are running.

3. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*
4. Insert tubes into the reaction module of the device
5. Run the amplification program
6. Analyze results after the amplification program is completed.

8.2.2.2. iQ

1. Program iQ according to manufacturer's manual and the Guidelines (see Tables 5-6).
2. Create a temperature profile on your iQ instrument as follows:

Table 5

iQ iCycler amplification program

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Repeats*
1	95	15 min	–	1
2	95	20 sec	–	45
	60	1 min	FAM, HEX	

Universal program**, AmpliSens-1 iQ**, can be used as well (see table 6). The program allows conducting any combination of tests (for example, for detection of DNA of sexually transmitted infections) in a single run.
Analytical performances of the reagents kits remain the same when applying universal amplification program.

Table 6

AmpliSens-1 iQ amplification program

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Повторов*
1	95 °C	15 min	–	1
2	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
3	95 °C	5 sec	–	40
	60 °C	30 sec	FAM, HEX, ROX, Cy5	
	72 °C	15 sec		

Note. ROX and Cy5 channels activates when necessary if multiplex format tests are running.

3. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*
4. Insert tubes into the reaction module of the device
5. Run the amplification program
6. Analyze results after the amplification program is completed.

8.2.2.3. Mx

1. Program Mx according to manufacturer's manual and the Guidelines (see Tables 7-8).
2. Create a temperature profile on your Mx instrument as follows:

Table 7

Mx3000P amplification program

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Repeats*
1	95	15 min	–	1
2	95	20 sec	–	45
	60	1 min	FAM, HEX/JOE	

Universal program**, AmpliSens-1 Mx**, can be used as well (see table 8). The program allows conducting any combination of tests (for example, for detection of DNA of sexually transmitted infections) in a single run.
Analytical performances of the reagents kits remain the same when applying universal amplification program.

Table 8

AmpliSens-1 Mx amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Repeats***
Segment 1	95	15 min	–	1
Segment 2 (Cycling)	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Segment 3 (Cycling)	95	5 sec	–	40
	60	30 sec	FAM, JOE, ROX, Cy5	
	72	15 sec		

Note. ROX and Cy5 channels activates when necessary if multiplex format tests are running.

3. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*
4. Insert tubes into the reaction module of the device
5. Run the amplification program
6. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

The character of providing analysis and calculating the results depend on the type of equipment.

Analysis of results is performed by software of the used real-time PCR instrument by measuring fluorescence signal accumulation in two channels:

– Accumulation of ***Gardnerella vaginalis*** **DNA** amplification product is detected in the channel for the FAM fluorophore.

– ***Lactobacillus*** **spp. DNA** is detected in **JOE/Yellow/HEX** channel.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at a specific level that corresponds to the presence (or absence) of a *Ct* value of a cDNA sample in the corresponding column of the result grid.

Principle of interpretation is the following:

– ***Gardnerella vaginalis*** **DNA** is **detected** if the *Ct* value determined in the result grid in the channel for the FAM fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

– ***Gardnerella vaginalis*** **DNA** is **not detected** in a sample if *Ct* value is not

determined (absent) in the channels for JOE fluorophores, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin*.

– The result is **invalid** if *Ct* value is not determined (absent) in the channel for JOE fluorophores, whereas the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or greater than the specified boundary Ct value. In such cases, PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.

– The result is **equivocal** if the *Ct* value determined in the channel for JOE fluorophore is greater than the boundary Ct value specified in the *Important Product Information Bulletin*, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin.* In such cases, PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained, the sample is considered positive.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

10. TROUBLESHOOTING

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

1. If any Ct value appears in FAM/Green channel (*Gardnerella vaginalis*) in the result grid for Negative Controls (C-, NCA) it indicates contamination of the reagents or samples. In this case results of the analysis for all samples are considered invalid. It is required to repeat the analysis of all tests, and to take measures to detect and eliminate the source of contamination.
2. If Calc Conc >50 appears in JOE/Yellow channel (*Lactobacillus* spp.) in the results grid for Negative Controls (C-, NCA) or if Calc Conc >5 at PCR stage it indicates contamination of the reagents or samples. In this case results of the analysis for all samples are considered invalid. It is required to repeat the analysis of all tests, and to take measures to detect and eliminate the source of contamination.
3. If concentration values of *Gardnerella vaginalis* and *Lactobacillus* spp. DNA for control samples ((BV-) and (BV+)) do not fall in a range it indicates the errors made during extraction or amplification stages. In this case it is necessary to repeat the PCR analysis.

11. TRANSPORTATION

AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT 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 / Lactobacillus* spp.-titre-FRT** PCR kit (except for Polymerase(TaqF) and PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp.) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT** 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.

Polymerase (TaqF) and PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp. are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp. is to kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Transport medium	Nucleic acid extraction kit	Microorganism	Sensitivity, GE/ml[1]
Discharge of posterior fornix of vagina	Transport Medium with Mucolytic Agent	DNA-sorb-AM	*Gardnerella vaginalis*	5×10^3
			Lactobacillus spp	5×10^3

The claimed analytical features of **AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT** PCR kit are guaranteed only when additional reagents kit “DNA-sorb-AM”, (manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”) is used.

13.2. Specificity.

Specificity of **AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT** PCR kit is ensured by selection of specific primers and probes, as well as the selection of stringent 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® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT** PCR kit was confirmed in laboratory clinical trials.

[1]Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.

13.3. Linear range of measurements.

Linear range of measurements for quantitative estimation of every identified microorganism is from 10^3 to 10^7 GE/ml.

14. REFERENCES

1. Handbook “Sampling, transportation, 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, 2008.

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* / *Lactobacillus* spp.-titre-FRT** 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
RUO	Research use only		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
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
26.12.10 LA	Cover page	The phrase “For Professional Use Only” 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 reagents before and after the first use was added
		Information that PCR-mix-1-FRT *Gardnerella vaginalis* / *Lactobacillus* spp. is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
03.07.11 RT	Cover page, text	The name of Institution was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
26.02.13 PE	Cover page, Key to symbols used	Symbol IVD was replaced with the symbol RUO
	Text	The name of Institution was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology” (3 times)
	Sensitivity	The table to describe analytical sensitivity was added
18.03.13 PE	Text	The subscript of PC was changed to (BV+) and (BV-)
29.03.13 PE	Text	The text was changed and enlarged according to the pattern
		The phrase “tips with aerosol barrier” was changed to “filter tips”
		The term “isolation” was changed to “extraction”
		Due to replacing Appendix 1 with the Guidelines, reference to the Appendix was substituted by reference to the Guidelines
	Principle of PCR detection	The sentence “Judge by analysis results (if all procedures are strictly followed) it can be estimated the vaginal microcenosis and diagnosed the bacterial vaginosis with high accuracy” was changed to “According to the analysis results (if all procedures are strictly followed) the vaginal microcenosis can be estimated and the bacterial vaginosis can be diagnosed with high accuracy.”
	Sampling and handling	The paragraph concerning transportation and storage conditions for the samples was added
	Amplification	The word “sce” was changed to “sec”
		The words “c” and “мин” were changed to “sec” and “min”
	Linear range of measurements	The paragraph was added