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

RUO

For Professional Use Only

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
RUO	Research use only		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
		BV-, BV+	Positive Controls of extraction

1. INTENDED USE

AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT PCR kit is not a medical device. PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Gardnerella vaginalis* and *Lactobacillus* species DNA in the biological material (discharge of posterior fornix of vagina) using real-time hybridization-fluorescence detection of amplified products.

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.

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

The method of *Lactobacillus* spp. and *Gardnerella vaginalis* DNA quantitative detection in biological material is based on:

a) Total DNA extraction from cell suspension.

NOTE: Biological material (discharge of posterior fornix of vagina) is to be placed into **Transport Medium with Mucolytic Agent** 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 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.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

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

Calculation of concentrations of *Gardnerella vaginalis* and *Lactobacillus* spp. DNA per 1 ml of a biological 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 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 biological material and the quantity of copies of the amplified gene in the genome of the microorganism.

Calculation of relation coefficient of *Lactobacillus* spp. DNA and *Gardnerella vaginalis* DNA 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

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	*Gardnerella vaginalis* DNA	*Lactobacillus* spp. DNA
Target gene	*gene* 16S rRNA	*gene* 16S rRNA

3. CONTENT

AmpliSens® *Gardnerella vaginalis / Lactobacillus* spp.-titre-FRT PCR kit is produced in 2 forms:

variant titre-FRT-100 F REF R-B7-FT(RG,iQ,Mx)-CE,

variant titre-FRT-100 F in bulk[1] REF R-B7-FT(RG,iQ,Mx)-CE-B.

Variant titre-FRT-100 F includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp.		clear liquid from colorless to light lilac colour	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

Variant titre-FRT-100 F 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 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 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany) iCycler iQ or iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator at the temperature from 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance 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 in the area where the previous step was performedreturn 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

NOTE: 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 for analysis of the DNA extracted with DNA extraction kits from the biological material (discharge of posterior fornix of vagina).

Biological material (0.05 ± 0.01 ml) should be obtained by universal probe or special applicator (Copan, Italy) and placed into 2 ml tube containing 0.5 ml of **Transport Medium with Mucolytic Agent**. Biological material volume should be 1/10 of transport medium volume. 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.

NOTE: Only one freeze-thaw cycle of biological 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

It is recommended to use **DNA-sorb-AM** nucleic acid extraction kits.

NOTE: Extract the DNA according to the manufacturer's protocol.

NOTE: Add **100 µl** of **Transport Medium with Mucolytic Agent** to the tube labeled C– (Negative Control of Extraction) during extraction procedure.

NOTE: To the Positive Control of extraction tubes ((BV-) and (BV+)) transfer **90 µl** of **Transport Medium with Mucolytic Agent** (per each) and **10 µl** of **Positive Control DNA *Gardnerella vaginalis / Lactobacillus* spp.-1** or **Positive Control 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. Prepare the mixture of PCR-buffer-FRT and Polymerase (TaqF). 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.

NOTE: The prepared mixture is intended for 120 samples.
The mixture can be stored at 2-8 ºC for 3 months and used as necessary.

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 2).

Table 2

Methods of reagents addition

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 mixture of PCR-buffer-FRT and Polymerase (TaqF)**	1. Prepare the reaction mixture for required number of reactions, calculating per each reaction: – **7 µl of PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp.** – **8 µl of prepared mixture 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 3). 2. Add **15 µl** of prepared mixture into the tubes.

Table 3

Scheme of reaction mixture 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
Mixture 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
Mixture 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.

NOTE: Avoid transferring of sorbent into reaction mixture 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

1. Create a temperature profile on your instrument as follows:

Table 4

Rotor-Gene 3000/6000 amplification program

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

NOTE: Universal program**, AmpliSens-1**, 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 5

AmpliSens-1 amplification program for rotor-type instruments

Step	Temperature, °C	Time	Fluorescence detection	Cycles
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.

Table 6

Amplification program for plate-type instruments

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

NOTE: 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 7

AmpliSens-1 amplification program for plate-type instruments

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

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

NOTE: If using CFX96 instruments set ***Ramp Rate* 2,5 °C/s** by clicking the ***Step Options*** button for each step of cycling.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

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

– The signal of the ***Gardnerella vaginalis* DNA** amplification product is detected in the channel for the FAM fluorophore.
– The signal of the ***Lactobacillus* spp. DNA** amplification product is detected in the channel for the JOE fluorophore.

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

The quantity of copies per reaction for *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA are calculated automatically by the software of the instrument using the specified calibrators values. The quantity of copies of *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA are given in the corresponding column of the result grid.

Using the *Ct* values and specified values of calibrators GL1, GL2, GL3 a calibration curve plotting and calculation of *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA copies per ml of initial clinical sample is performed automatically:

NOTE: The *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA concentrations for BV- and BV+ control samples are to be in the range specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

10. TROUBLESHOOTING

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

1. If any *Ct* value appears for Negative Control of extraction and Negative Control of amplification in the channel for the FAM fluorophore (*Gardnerella vaginalis*) in the result grid 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 for Negative Control of extraction and/or if ***Calc Conc*** >5 appears for Negative Control of amplification in the channel for the JOE fluorophore (*Lactobacillus* spp.) in the result grid 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 specified in the *Important Product Information Bulletin* 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 stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: 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.

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

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Transport medium	Nucleic acid extraction kit	Microorganism	Sensitivity, copies/ml[2]
Discharge of posterior fornix of vagina	Transport Medium with Mucolytic Agent	DNA-sorb-AM	*Gardnerella vaginalis*	$5x10^3$
			Lactobacillus spp	$5x10^3$

13.2. Specificity

The analytical 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.

Nonspecific responses were absent in tests of human DNA samples and the panel of the following microorganisms DNA samples: *Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Candida glabrata, Candida krusei, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Chlamydia trachomatis, Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV types* 1 and 2, *CMV, HPV* and human DNA.

13.3. Linear measurement range

The linear measurement range for quantitative estimation of each detected microorganism is from 10^3 to 10^7 copies/ml.

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.
2. Guidelines to the **AmpliSens® *Gardnerella vaginalis* / *Lactobacillus* spp.-titre-FRT** PCR kit for qualitative and quantitative detection of *Gardnerella vaginalis* and *Lactobacillus* species DNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection.

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.

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
05.11.14 ME	Text	Text was corrected in accordance with the template and Russian instruction manual
	13. Specifications	GE/ml was changed to copies/ml
14.05.15 PM	Text	Clinical material was changed to biological
	1. Intended use	The phrase "The results of PCR analysis are taken into account in complex diagnostics of disease" was changed to "For research use only. Not for diagnostic procedures"
	13.2. Specificity	The phrase "Specificity of AmpliSens® *Gardnerella vaginalis* / *Lactobacillus* spp.-titre-FRT PCR kit was confirmed in laboratory clinical trials" was deleted.
19.06.18 PM	3. Content	The colour of the reagent was specified
02.08.18 EM	2. Principle of PCR detection	The information about the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glicosylase (UDG) and deoxyuridine triphosphate was added
23.06.20 KK	Through the text	The text formatting was changed
	Footer	The phrase "For research use only. Not for diagnostic procedures" was added
04.08.23 BA	Footer	The REF R-B7-FT(RG,iQ,Mx)-CE-B was added
	3. Content	Variant titre-FRT-100 F in bulk was added
	12. Stability and storage	Information that PCR-mix-1-FRT *Gardnerella vaginalis* / *Lactobacillus* spp. is to be kept away from light was added

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[2]The quantity of the pathogen agent DNA copies per 1 ml of a clinical sample, placed into the specified transport medium.