RUO

For Professional Use Only

AmpliSens® *Florocenosis I*

Bacterial vaginosis-FRT

PCR kit

Instruction Manual

AmpliSens®

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT PCR kit is an *in vitro* nucleic acid amplification test for diagnosing bacterial vaginosis (quantitation of DNA of *Gardnerella vaginalis, Atopobium vaginae*, *Lactobacillus* spp., and total amount of bacteria) in clinical materials by using real-time hybridization-fluorescence detection.

This PCR kit allows assessment of the ratio between the total number of bacteria, lactobacteria, and opportunistic pathogenic bacteria associated with bacterial vaginosis (*Gardnerella vaginalis, Atopobium vaginae*) in the vaginal biotope. Determination of the total number of bacteria makes it possible to assess the adequacy of collected samples. As the material for PCR serves vagina secretion DNA and epithelial cells scrape from the vagina side area.

The ratio between the logarithms of concentrations of *Lactobacillus* spp. and the total amount of bacteria, the ratio between the logarithms of concentrations opportunistic pathogenic microbial flora (*Gardnerella vaginalis* and *Atopobium vaginae)* and total amount of bacteria and the ratio between the logarithms of concentrations of *Lactobacillus* spp. and opportunistic pathogenic microbial flora (*Gardnerella vaginalis* and *Atopobium vaginae)* allows diagnosing bacterial vaginosis with a high accuracy. Bacterial vaginosis is a condition associated with the suppression of normal microbial vaginal flora (*Lactobacillus* spp.) and its replacement with opportunistic pathogenic bacteria (including *Gardnerella vaginalis* and *Atopobium vaginae*).

The analysis performed with the use of the **AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT** PCR kit allows dynamic monitoring of the state of the vaginal biotope and to control the treatment effectiveness.

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

2. PRINCIPLE OF PCR DETECTION

The assessment of the state of vaginal microbiocenosis (quantitation of DNA of *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp., and the total amount of bacteria) is based on:

1. Total DNA extraction from the clinical sample (vaginal swab containing vaginal epithelial cells or vaginal discharge) placed in 0.5 ml of Transport Medium with Mucolytic Agent.

2. Simultaneous amplification (multiprime PCR) of DNA fragments of *Gardnerella vaginalis, Atopobium vaginae*, *Lactobacillus* spp., and DNA of total amount of bacteria with real-time hybridization-fluorescence detection.

In the content of the reaction mixture fluorescently marked oligonucleotide probes which hybridizate with complementary part of DNA-target present. As a result the fluorescence intensity increases. This fact allows to register the accumulation of specific amplification product measuring the intensity of fluorescence signal. The detection of fluorescent signal is carried out during the PCR with the help of thermocycler with the fluorescent signal "real-time" detection system.

To quantify the number of copies of DNA of *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp., and total amount of bacteria in a standard sample volume, quantitative standards (calibrators) that allow quantifying the number of copies of DNA of *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp., and the total amount of bacteria in a reaction tube are used.

3. CONTENT

AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT PCR kit is produced in 2 forms:

AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT PCR kit variant FRT-100 F (for use with RG) **REF** R-B74-100-FT(RG)-CE.

AmpliSens® Florocenosis / Bacterial vaginosis-FRT PCR kit variant FRT-100 F in bulk[1] (for use with RG) **REF** R-B74-100-FT(RG)-CE-B.

AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT PCR kit variant FRT-100 F includes:

Reagent		*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis		colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT		colorless clear liquid	0.6	1 tube
Polymerase (TaqF)		colorless clear liquid	0.06	1 tube
DNA-buffer		colorless clear liquid	0.5	1 tube
DNA calibrators	**FC1**	colorless clear liquid	0.4	1 tube
	FC2	colorless clear liquid	0.4	1 tube
Positive Control BV+ ($C+_{BV+}$)*		colorless clear liquid	0.1	1 tube
Positive Control BV– ($C+_{BV-}$)*		colorless clear liquid	0.1	1 tube

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

AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT PCR kit is intended for

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

110 reactions (including controls and calibrators).

Enclosed in PCR kit:

1. Compact disk with **“AmpliSens® *Florocenosis* / Bacterial vaginosis”** program (Microsoft Excel) for processing of data and generation of results.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Additional materials and equipment for DNA extraction.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with filters (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, Rotor-Gene Q (Qiagen, Germany), iCycler iQ, iCycler iQ5 (Bio-Rad, USA)).
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 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, 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, or 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 DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then 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 the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® ***Florocenosis*** **/ Bacterial vaginosis-FRT** PCR kit is intended for the analysis of DNA extracted from vaginal discharge and vaginal epithelial cells (females only).

As the examinated material serve vaginal scrapes (epithelial cells scrapes from the vagina side area). The obtaining of the material should be made with the help of the plastic probe or swab into a tube with 2 ml of Transport Medium with Mucolytic Agent. Clinical material is to be collected in a sufficient amount. To collect clinical material insert the probe in posterior vaginal vault and rotating the probe swipe the epithelium.

Transfer the probe to a tube with the Transport Medium with Mucolytic Agent. Break off the working part of the probe and leave it in the tube. If the amount of the collected material is sufficient, transport medium becomes muddy and changes color from pink to lemon-yellow in case the vaginal discharge pH is acid. The color may remain the same or slightly

change if the vaginal discharge pH is >4.5. If the tube with transport medium already contains the material taken from the cervix (cervical mucus), then the addition of the vaginal discharge with an acid pH (<4.5) does not change the color because the cervical mucus alkalifies the medium. Then, tightly close the tube, label it, and send to a laboratory for processing. The self-taken material got in the accordance with the instruction manual to the "Kit for self-taking vaginal scrape for microscopical investigation and infection diagnosis with PCR" can also be examined.

Storage of collected material:

- at 20–22 °C for 48 h,
- at 2–8 °C for 2 weeks.

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

7. WORKING CONDITIONS

AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT 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** K1-12-100-CE,
- Addition reagent, Transport Medium with Mucolytic Agent, **REF** 952-CE, is required.

Extract DNA according to the manufacturer's instructions.

Addition of Internal Control sample is not required!

1. To the tube intended for **Negative Control of Extraction (C–)** add 100 µl of Transport Medium with Mucolytic Agent
2. To the tube intended for **Positive Control of Extraction (BV–)**

add **10 µl** of **Positive Control BV–** and **90 µl** of **Transport Medium with Mucolytic Agent**

3. To the tube intended for **Positive Control of Extraction (BV+)**

add **10 µl** of **Positive Control BV+** and **90 µl** of **Transport Medium with Mucolytic Agent**

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

The choice of the amplification tubes depends on the thermocycler with the “real-time” detection system.

To put reagents, DNA probes and control samples into the tubes one should use the tips with filters.

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

Prepare the reaction mixture straight before the test. Reagents should be mixed in the following proportion (given volumes are calculated for one reaction):

- **10 µl of PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis.**
- **5 µl of mixture of PCR-mix-2-FRT and polymerase (TaqF).**

1. It is necessary to prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. Transfer the entire content of one tube with **polymerase (TaqF) (60 µl)** to the tube with **PCR-mix-2-FRT (600 µl)**. Avoid foaming while vortexing the tube. Indicate the mixture preparation date on the tube.

The prepared mixture is intended for analysis of 120 samples. The mixture should be stored at 2–8 °C for up to 3 months and used as required.

If the mixture cannot be utilized within 3 months, it should be prepared for a smaller number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and **15 µl of polymerase (TaqF).** Thus prepared mixture is intended for 30 reactions.

2. Thaw and vortex the tube with **PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis.** Centrifuge shortly to remove the drops from the caps of the tubes.

Calculate the required number of reactions including the test and control samples according to Appendix 1. Note that even for analysis of one test DNA sample in the quantitative format, it is necessary to run **5 controls of the amplification stage**: **2 DNA-calibrators (FC1 and FC2) in two repeats, and the Negative Control of Amplification (DNA-buffer)**.

It is necessary to take reagents for one extra reaction: for N tests, prepare reagents for (N+1) reactions.

3. Prepare the reaction mixture in an individual tube. Mix **PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis and mixture of PCR-mix-2-FRT with polymerase (TaqF)** prepared as described in point 1 of Section 8.2.1.
4. Prepare the required number of tubes for amplification of DNA from clinical and control samples.

5. Transfer **15 µl** of prepared reaction mixture to the tubes.
6. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes.

Avoid sorbent adding when transferring the DNA samples.

7. Carry out the control reactions:

NCA	- Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
DNA calibrator FC1 **DNA calibrator FC2**	- Add **10 µl** of **DNA calibrator FC1** and **10 µl** of **DNA calibrator FC2** to two tubes labeled FC1 and FC2, consequently
C–	- Add **10 µl** of the sample extracted from **Transport Medium with Mucolytic Agent** to the tube labeled C– (Negative Control of Extraction).
BV–	– add **10 µl** of the sample extracted from **Positive Control BV–** to the tube labeled BV–
BV+	– add **10 µl** of the sample extracted from **Positive Control BV+** to the tube labeled BV+

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

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

Table 1

AmpliSens-1 amplification program for rotor-type instruments[2]

Step	***Temperature, °C***	***Time***	***Fluorescent signal detection***	***Cycle repeats***
Hold	95	15 min	-	1
Cycling	95	5 s	-	5
	60	20 s	-	
	72	15 s	-	
Cycling 2	95	5 s	-	40
	60	20 s	FAM/Green, JOE/Yellow ROX/Orange, Cy5/Red	
	72	15 s		

[2] For example Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (Qiagen, Germany).

Table 2

AmpliSens-1 amplification program for plate-type instruments[3]

Step	*Temperature, °C*	*Time*	*Fluorescent signal detection*	*Cycle repeats*
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	95	5 s	–	40
	60	30 s	FAM, JOE/HEX, ROX, Cy5	
	72	15 s	–	

4. Insert tubes into the reaction module of the device.
5. Run the amplification program with fluorescence detection.
6. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

The results are interpreted by the software of the used real-time PCR cycler. Curves of fluorescent signal accumulation are detected in four channels:

- ***Gardnerella vaginalis* DNA** is detected in the **FAM/Green** fluorescence channel,
- ***Atopobium vaginae* DNA** is detected in the **JOE/Yellow** fluorescence channel,
- ***Lactobacillus* spp. DNA** is detected in the **ROX/Orange** fluorescence channel,
- **Total bacteria DNA** is detected in the **Cy5/Red** fluorescence channel.

Cycle threshold *(Ct)* is a cycle when fluorescence curve crosses the threshold line. Cycle threshold values are analyzed by the program of automatic result interpretation. On the basis of *Ct* values and preset values of DNA calibrators, FC1 and FC2, calibration line is plotted and calculation of DNA copies of *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp., and total bacteria is performed.

Concentration values of DNA calibrators are specified in the *Important Product Information Bulletin* for each lot of the PCR kit. They should be entered in the corresponding cells of automatic interpretation program.

Principle of interpretation is the following:

- the result of a sample is considered **positive** in the **FAM/Green, JOE/Yellow, ROX/Orange,** and **Cy5/Red** channels if fluorescence curve is S-shaped and crosses the threshold line at the area of reliable fluorescence growth.

[3] For example iCycler iQ, iQ5 (Bio-Rad, USA).

– the result of a sample is considered **negative** in the **FAM/Green, JOE/Yellow, ROX/Orange,** and **Cy5/Red** channels if fluorescence curve does not cross the threshold line (*Ct* value is absent) and does not have typical S-shape.

– the result of a sample is considered **unreliable** if the signal in the **Cy5/Red** channel is absent or when ***Calc Conc*** value of an analyzed sample in the **Cy5/Red** channel is less than 1000 copies/reaction.

– the result of a sample is considered **invalid** if the signal in the **Cy5/Red** channel is absent (no *Ct* value) or if ***Calc Conc*** value of an analyzed sample in the **ROX/Orange** channel is **greater** than ***Calc Conc*** value in the **Cy5/Red channel by 0.5 log.**

Boundary concentration values of control samples are specified in the *Important Product Information Bulletin* enclosed in PCR kit.

The results of analysis are considered reliable only if the results obtained for control samples, C–, (BV–), (BV+), and NCA, are correct (see table 3) and for DNA calibrators, FC1 and FC2, *Ct* values are detected.

Table 3

Results for controls

Control	**Stage for control**	**Result of amplification in channel, copies/reaction**			
		FAM /Green	**JOE/ Yellow**	**ROX/ Orange**	**Cy5/ Red**
C–	DNA extraction	< boundary *Ct* value*	< boundary *Ct* value	< boundary *Ct* value	< boundary *Ct* value
BV–	DNA extraction	< boundary *Ct* value	< boundary *Ct* value	> boundary *Ct* value	> boundary *Ct* value
BV+	DNA extraction	> boundary *Ct* value	> boundary *Ct* value	< boundary *Ct* value	> boundary *Ct* value
NCA	Amplification	< boundary *Ct* value	< boundary *Ct* value	< boundary *Ct* value	< boundary *Ct* value
FC1, FC2	Amplification	*Ct* value and ***Calc Conc*** are determined	*Ct* value and ***Calc Conc*** are determined	*Ct* value and ***Calc Conc*** are determined	*Ct* value and ***Calc Conc*** are determined

Interpretation of results

The ratio coefficients, RC1 and RC2, which are used for interpretation of data for clinical and control samples, as well as the concentrations of DNA of *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp., and the total bacterial DNA are calculated automatically with the help of Microsoft Excel according to the instruction. The algorithm of obtaining data is described in Guidelines to **AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT** PCR kit for diagnosing bacterial vaginosis (quantitation of DNA of *Gardnerella vaginalis, Atopobium vaginae*, *Lactobacillus* spp., and total amount of bacteria) in the clinical material by using real-time hybridization-fluorescence detection.

10. TROUBLESHOOTING

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

1. If the **Calc Conc (copies/reaction)** value greater than 5 appears in the results grid for the Negative Control of extraction (C–) and/or Negative Control of PCR (NCA) in the FAM/Green and/or JOE/Yellow channel, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. The analysis must be repeated starting with the extraction stage for the samples that showed the presence of *Gardnerella vaginalis* and/or *Atopobium vaginae* DNA. Measures to detect and eliminate the source of contamination must also be taken.

2. If the values (copies/reaction) for the FC1 and FC2 calibrator differ from the specified values by more than 30%, check the tube order in the rotor. For rotor-type instruments, well No. 1 should be loaded with a test tube (should not be vacant)).

3. If the value of the Correlation Coefficient, R^2, is less than 0.9, it indicates calibration failure. Ensure that calibrators are set correctly and fix inaccuracies. If this does not help, repeat PCR for all samples and calibrators.

4. If the *Ct* value of the Positive Control of extraction “BV–” is absent in the ROX/Orange or Cy5/Red channels, the results of all samples are considered invalid. Repeat PCR for all samples.

5. If the *Ct* value of the Positive Control of extraction “BV+” is absent in one or more channels (FAM/Green, JOE/Yellow, ROX/Orange, or Cy5/Red), the results for all samples are considered invalid. Repeat PCR for all samples.

6. If a signal of a test sample is absent in the **Cy5/Red** channel or if the **Calc Conc** value in the **Cy5/Red** channel is less than 10 000 copies/reaction, the result is considered as unreliable and PCR should be repeated for that sample. If the same result is

reproduced, sampling of the clinical material should be repeated.

Unreliable result can be obtained in the following cases:

- insufficient amount or poor quality of collected material;
- woman receives antibacterial therapy. Repeat the test in no sooner than two weeks after the end of therapy;
- material was taken from a prepubertal girl (before menarche) or from a woman in menopause;
- material belongs to a man.

7. If the signal of a test sample is absent (*Ct* value is absent) in the **Cy5/Red** channel or if the quantity of *Lactobacillus* spp. is greater than the total quantity of bacteria by more than 0.5 Log, the result for this sample is considered **invalid**. The analysis of the sample should be repeated starting from the extraction stage. If the same result is reproduced, sampling of the clinical material should be repeated.

11. TRANSPORTATION

AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT** PCR kit (except for PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis, polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens®** Florocenosis **/ Bacterial vaginosis-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis, PCR-mix-2-FRT, polymerase (TaqF)	are to be stored at temperature from minus 24 to minus 16 °C when not in use

PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® Florocenosis / Bacterial vaginosis-FRT** PCR kit is the following:

Clinical material	Transport medium	Nucleic acid extraction kit	PCR kit	Sensitivity, copies/ml
Epithelial cells of vagina	Transport Medium with Mucolytic Agent	DNA-sorb-AM	PCR kit variant FRT-100 F	$5x10^3$
Vaginal discharge	Transport Medium with Mucolytic Agent	DNA-sorb-AM	PCR kit variant FRT-100 F	$5x10^3$

13.2. Specificity

PCR kit detects *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp. DNA and bacteria DNA. The clinical specificity of the kit is proved by the clinical material examination with the following results confirmation by the sequence analysis of the amplification fragments.

Nonspecific reactions were absent during testing of human DNA samples and DNA panels of the following microorganisms: *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*-1 and *HSV*-2, *CMV*, and *HPV.*

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 to *Florocenosis* / Bacterial vaginosis-FRT PCR kit for diagnosing bacterial vaginosis (quantitation of DNA of *Gardnerella vaginalis, Atopobium vaginae*, *Lactobacillus* spp., and total amount of bacteria) in the clinical materials by using real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology.

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®** ***Florocenosis* / Bacterial vaginosis-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	Σ	Sufficient for
RUO	Research use only		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of Amplification

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
22.03.12 LA	4. Additional requirements	Real-time instruments, Rotor-Gene Q (Qiagen, Germany), iCycler iQ, iCycler iQ5 (Bio-Rad, USA) are added
	8.2.2. Amplification	AmpliSens-1 amplification program for plate-type instruments is added
	9. Data analysis	Interpretation of results is edited. Table 4 and 5 are added
	10. Troubleshooting	Paragraphs 4, 5, 6, 7 are corrected
17.05.12 Ivl	Title page, Key to symbols used	Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only
	Content	DNA calibrators FC1 and FC2 volume was changed from 0.2 ml to 0.4 ml
27.03.13 PE	Text	The names of the PCR kit and PCR-mix-1 were renewed
	Intended use, principle of PCR detection, sampling and handling, protocol, data analysis, specifications	The paragraphs were renewed in accordance with the Russian version
	Additional requirements	"Additional materials and equipment for DNA extraction" were added
	Footer	Catalogue number R-B74-100-FT(RG)-CE-B was deleted
14.08.13 FN	Footer	Catalogue number **REF** R-B74-100-FT(RG)-CE-B was added
21.08.13 FN	Content	One more release form was added: AmpliSens® Florocenosis / Bacterial vaginosis-FRT PCR kit variant FRT-100 F in bulk (for use with RG) **REF** R-B74-100-FT(RG)-CE-B
09.10.13 GA	Text	The name was changed from "AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT" to "AmpliSens® Flora-BV"
	4. Additional requirements	The phrase "Deep-freezer for ≤ –16 °C" was changed to "Deep-freezer at the temperature from minus 24 to minus 16 °C"
11.11.13 GA	Text	The name was changed from "AmpliSens® Flora-BV" to "AmpliSens® *Florocenosis* / Bacterial vaginosis-FRT"