CE

IVD

For Professional Use Only

Florocenosis /

Bacterial vaginosis-FRT

PCR kit

Instruction Manual

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1. INTENDED USE

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 the clinical material using real-time hybridization-fluorescence detection.

This PCR kit allows assessment of the ratio between the total amount of bacteria, lactobacteria, and opportunistic pathogenic bacteria associated with bacterial vaginosis (*Gardnerella vaginalis, Atopobium vaginae*) in the vaginal biotope. Determination of the total amount 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 of opportunistic pathogenic microbial flora (*Gardnerella vaginalis* and *Atopobium vaginae)* and the 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 **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 **REF** 952-CE; **REF** 953-CE.

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 real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

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

Florocenosis / Bacterial vaginosis-FRT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase by chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

Florocenosis / Bacterial vaginosis-FRT PCR kit is produced in 1 form:

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

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+*		colorless clear liquid	0.1	1 tube
Positive Control BV–*		colorless clear liquid	0.1	1 tube

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

Florocenosis / Bacterial vaginosis-FRT PCR kit is intended for 110 reactions (including controls and calibrators).

Enclosed in PCR kit:

1. Compact disk with the software in Microsoft Excel format for processing of data and

generation of results.

4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (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).
  a) 0.2-ml PCR tubes with domed 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 for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

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 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.

Florocenosis / Bacterial vaginosis-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (vaginal discharge and vaginal epithelial cells (females only)).

The material should be obtained by the universal or cotton swab into a 2-ml tube with Transport Medium with Mucolytic Agent **REF** 952-CE; **REF** 953-CE. Clinical material is to be collected in a sufficient amount. Deep the swab into the discharge of the posterior vaginal vault. Turn the swab while rubbing it against the surface of epithelium. Collect as much of the material as possible by the swab.

Transfer the swab into a tube with the Transport Medium with Mucolytic Agent. Break off the lower part of the swab and leave it in the tube with transport medium. 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 cap the tube avoiding an airspace formation and deformation of the interior part of the cap. Mark the tube.

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.

Before the DNA extraction, make sure that the swabs are leaved in the tubes with the test material, the tubes are tightly closed and the transport medium is present in the tubes with the clinical material in a sufficient quantity (no less than 500 µl).

The samples should be thawed before the DNA extraction (if the samples were stored long time), vortex the tubes thoroughly and sediment the drops from the caps by short centrifugation.

7. WORKING CONDITIONS

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 the DNA according to the manufacturer's protocols.

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 type of tubes depends on the type of PCR real-time instrument.

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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)** into the tube with **PCR-mix-2-FRT (600 µl)**. Vortex the tube avoiding foaming. 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 necessary.

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 Table 1. Note that even for analysis of one test DNA sample in the quantitative format, it is necessary to carry out **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.

Table 1

Scheme of reaction mixture preparation

	Reagent volume for specified number of reactions, µl	
Reagent volume per one reaction, µl	**10,0**	**5,0**
Number of reactions for quantitative detection (including 3 controls of extraction)	**PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis[1]**	**Mixture of PCR-mix-2-FRT and polymerase (TaqF)***
4	**100**	**50**
5	**110**	**55**
6	**120**	**60**
7	**130**	**65**
8	**140**	**70**
9	**150**	**75**
10	**160**	**80**
11	**170**	**85**
12	**180**	**90**
13	**190**	**95**
14	**200**	**100**
15	**210**	**105**
16	**220**	**110**
17	**230**	**115**
18	**240**	**120**
19	**250**	**125**
20	**260**	**130**
21	**270**	**135**
22	**280**	**140**
23	**290**	**145**
24	**300**	**150**
25	**310**	**155**
30	**360**	**180**

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. Take the required number of tubes for amplification of DNA obtained from clinical and control samples.
5. Transfer **15 µl** of prepared reaction mixture into the tubes.
6. Add **10 µl** of **DNA** obtained at the DNA extraction stage to the prepared tubes.

[1] The values are specified with account of one extra reaction and with account of carrying out 5 controls of amplification stage (2 DNA-calibrators FC1 and FC2 (in two repeats) and negative control (DNA-buffer))

Avoid transferring of sorbent together with 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** to two tubes labeled FC1 and **10 µl** of **DNA calibrator FC2** to the other two tubes labeled FC2.
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– (Positive Control of Extraction)
BV+	– add **10 µl** of the sample extracted from **Positive Control BV+** to the tube labeled BV+ (Positive Control of Extraction)

8.2.2. Amplification

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

Table 2

	***Rotor-type instruments*[2]**			***Plate-type instruments*[3]**		
Step	***Temperature, °C***	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
1	95	15 min	1	95	15 min	1
2	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
3	95	5 s	40	95	5 s	40
	60	20 s Fluorescence acquiring		60	30 s Fluorescence acquiring	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX and Cy5 fluorophores.

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 four channels:

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

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

- The signal of the *Gardnerella vaginalis* DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *Atopobium vaginae* DNA amplification product is detected in the channel for the JOE fluorophore.
- The signal of the *Lactobacillus* spp. DNA amplification product is detected in the channel for the ROX fluorophore.
- The signal of the total bacteria DNA amplification product is detected in the channel for the Cy5 fluorophore.

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 channels for the **FAM, JOE, ROX** and **Cy5** fluorophores if fluorescence curve is S-shaped and crosses the threshold line at the area of reliable fluorescence growth.
- the result of a sample is considered **negative** in the channels for the **FAM, JOE, ROX** and **Cy5** fluorophores 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 channel for the **Cy5** fluorophore is absent or when ***Calc Conc*** value of an analyzed sample in the channel for the **Cy5** fluorophore is less than 1000 copies/reaction (that corresponds to 10^5 copies/ml).
- the result of a sample is considered **invalid** if the signal in the channel for the **Cy5** fluorophore is absent (no *Ct* value) or if ***Calc Conc*** value of an analyzed in the channel for the **ROX** fluorophore is **greater** than ***Calc Conc*** value in the channel for the **Cy5** fluorophore **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 for the fluorophore			
		FAM	**JOE**	**ROX**	**Cy5**
C–	DNA extraction	< boundary value	< boundary value	< boundary value	< boundary value
BV–	DNA extraction	< boundary value	< boundary value	> boundary value	> boundary value
BV+	DNA extraction	> boundary value	> boundary value	< boundary value	> boundary value
NCA	PCR	< boundary value	< boundary value	< boundary value	< boundary value
FC1, FC2	PCR	*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

The ratio coefficients, 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 using the software in Microsoft Excel format according to the instruction. The algorithm of obtaining data is described in Guidelines [2].

10. TROUBLESHOOTING

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

1. If the ***Calc Conc*** value greater than 5 copies/reaction (that corresponds to 500 copies/ml) appears in the results grid for the Negative Control of extraction (C–) and/or Negative Control of PCR (NCA) in the channels for the FAM and/or JOE fluorophores, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. The analysis must be repeated from the extraction stage for all in which *Gardnerella vaginalis* and/or *Atopobium vaginae* DNA was detected. The measures for detection and elimination of contamination source should be assumed.
2. If the values (copies/reaction) of FC1 and FC2 calibrators differ from the specified ones by more than 30%, check the tubes order in the instrument. For rotor-type instruments well 1 must be filled with any tube containing reaction mix.
3. If the value of the correlation coefficient, R^2, is less than 0.9, calibration failure has occurred. Make sure that calibrators are set correctly and correct if necessary. If it 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 channels for

the ROX or Cy5 fluorophores, the results of analysis are considered invalid for all samples. Repeat PCR for all the samples.

5. If the *Ct* value of the Positive Control of extraction (BV+) is absent in one or more channels (for the FAM, JOE, ROX, or Cy5 fluorophores), the results of analysis are considered invalid for all samples. Repeat PCR for all the samples.
6. If a signal of a test sample is absent in the channel for the Cy5 fluorophore or if ***Calc Conc*** value in the in the channel for the Cy5 fluorophore is less than 10^5 copies/ml, the result is considered unreliable and PCR should be repeated for this sample. If the same result is reproduced, re-sampling is recommended.
7. If the signal of a sample is absent (*Ct* value is absent) in the channel for the Cy5 fluorophore 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, re-sampling is recommended.

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

11. TRANSPORTATION

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 **Florocenosis / Bacterial vaginosis-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FRT *Florocenosis* / Bacterial vaginosis, polymerase (TaqF) and PCR-mix-2-FRT). All components of the **Florocenosis / Bacterial vaginosis-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.

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.

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

13. SPECIFICATIONS

13.1. Sensitivity

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

Clinical material	Transport medium	Nucleic acid extraction kit	PCR kit	Sensitivity, copies/ml
Epithelial cells scrapes from the lateral walls 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

The analytical specificity of **Florocenosis / Bacterial vaginosis-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

PCR kit detects *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp. DNA and total 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.*

The clinical specificity of **Florocenosis / Bacterial vaginosis-FRT** PCR kit was confirmed in laboratory clinical trials.

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 material 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 **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		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	**BV–, BV+**	Positive controls of extraction
		FC1, FC2	DNA calibrators

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
19.05.15 ME	Text	Corrections according the template and Russian instruction manual
	8.2.1. Preparing tubes for PCR	Appendix 1 was integrated into the text of the instruction manual as Table 1
	9. Data analysis	For unreliable result the boundary value translated into copies/ml was added. In the table “Results for controls” the units for amplification results were deleted
	10. Troubleshooting	For Negative Control of extraction (C–) and/or Negative Control of PCR (NCA) the translation into copies/ml was added