RUO

Research Use Only

GUIDELINES

to 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

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

For research use only. Not for diagnostic procedures

TABLE OF CONTENTS

INTENDED USE

The guidelines describe the procedure of using **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 (discharge of posterior fornix of vagina) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection with the use of the following instruments:

- Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia),
- Rotor-Gene Q (QIAGEN, Germany),
- iCycler iQ, iCycler iQ5 (Bio-Rad, USA),
- Mx3000P and MX3005P (Stratagene, USA),
- CFX96 (Bio-Rad, USA).

AMPLIFICATION AND DATA ANALYSIS USING Rotor-Gene 3000/6000 (Corbett Research, Australia) AND Rotor-Gene Q (QIAGEN, Germany) INSTRUMENTS

When working with Rotor-Gene 3000 one should use the Rotor-Gene Version 6 software and the Rotor-Gene 6000 versions 1.7 (build 67) software or higher for Rotor-Gene 6000 and Rotor-Gene Q instruments.

Hereinafter, all the terms corresponding to different instruments and software are indicated in the following order: for Rotor-Gene 3000 / for Rotor-Gene 6000.

When carrying out the amplification it is recommended to use transparent PCR tubes (0.2 ml) with flat caps (detection through the bottom of the tube), or 0.1 ml tubes.

Programming the thermocycler

1. Switch the instrument on.
2. Insert the tubes into the rotor so that the first tube is in the Well 1, put the rotor into the instrument and close the lid (the wells of the rotor are numbered, these numbers are used for further programming of samples position in the instrument). The tubes are to be inserted into the rotor's wells in the following order: at first test samples, then control samples (BV-) and (BV+), then negative controls and finally DNA-calibrators – GL1, GL2, GL3.

NOTE: Balance the rotor of the instrument if it is not loaded entirely. Fill the spare wells with empty tubes (don't use the tubes left after previous experiments). Well 1 must be filled with any studied tube except for an empty one.

3. Activate the ***New*** button in the main menu.
4. In the open window select ***Advanced*** and ***Dual Labeled Probe/Hydrolysis probes***. Click the ***New*** button.
5. Select **36-Well Rotor** or **72-Well Rotor** and tick ***No domed 0.2 ml Tubes*** (Rotor-Gene

3000)/***Locking ring attached*** (Rotor-Gene 6000). Click ***Next***.

6. In the open window select operator and choose ***Reaction volume*** – **25 µl**. Make sure that the ***15 µl oil layer volume*** box is ticked. Click ***Next***.
7. Activate the ***Edit profile*** button and create a temperature profile (see tables 1, 2)

Table 1

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 RG**, can be used as well (see table 2). The program allows conducting any combination of tests (for example, for detection of DNA of sexually transmitted infections) in a single run.

Table 2

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	
	72	15 sec	–	

8. After the temperature profile has been selected click ***OK*****.**
9. Select the ***Calibrate/Gain Optimisation*** button in the ***New Run Wizard*** window, calibrate in the FAM/Green, JOE/Yellow channels.
   - activate the ***Calibrate Acquiring/Optimise Acquiring*** button;
   - to set channels calibration, indicate **5** in the ***Min Reading*** box and 8 **in the *Max Reading* box.** Activate the ***Perform Calibration Before 1st Acquisition/ Perform Optimisation Before 1st Acquisition*** button**.** Click ***Close*****.**
10. Click ***Next***, start the amplification program by activating the ***Start Run*** button.
11. Name the experiment and save it on a disk (results of the run will be automatically saved in this file). In the **Name** column define name/numbers of clinical samples and controls.
12. Enter the data into the grid of the samples (it opens automatically after the amplification has been started). Set the type ***Unknown*** opposite all the test samples. Set the type ***Standard*** opposite calibrators select the ***copies/reaction*** units**.** Set the type ***None*** for the wells matching with the corresponding empty tubes.

NOTE: Samples indicated as ***None*** won't be analysed.

13. Due to the use of mixed calibrators which contain several DNA templates (*Gardnerella vaginalis and Lactobacillus* spp.) it is necessary to use several pages to set DNA-calibrators concentrations. In the ***Page*** field in the ***Name*** column specify ***Gv*.** Insert calibrators values for *Gardnerella vaginalis* DNA on the first page (Gv). Click ***New*.** The new page for setting samples will appear. The names of the samples are set automatically like on the previous page, so the type ***Standard*** for calibrators should be set and enter **GL1, GL2, GL3** values in the ***Given Conc*** column for *Lactobacillus* spp.DNA (channel JOE/Yellow). In the ***Page*** field in the ***Name*** column enter **Lsp.**

Proceed to the results analysis at the end of the program.

Data analysis of *Gardnerella vaginalis* DNA amplification (FAM/Green channel)

1. Activate the button ***Analysis*** in the menu, select the mode of the analysis ***Quantitation***, activate the button **Cycling A FAM** or **Cycling A Green**, **Show**. In the ***Select the sample page to use*** select ***Gv***.
2. Cancel the automatic choice ***Threshold***.
3. Activate the ***Dynamic tube*** and **Slope Correct** buttons in the main window menu.
4. Activate the ***More Settings*/*Outlier Removal*** button and enter set **10 %**.
5. In ***CT Calculation*** menu set ***Threshold* = 0.05.**
6. In the ***Calc. Conc.*** column of the ***Quant. Results*** window the number of *Gardnerella vaginalis* DNA copies in a PCR-sample will appear.
7. Note, that in the ***Standard Curve*** window, the R coefficient (coefficient of correlation) should be no less than 0.99. The ***Efficiency*** should fit in 0,85 – 1,08 range.

Data analysis of *Lactobacillus* spp. DNA amplification (JOE/Yellow channel)

1. Activate the button ***Analysis*** in the menu, select the mode of the analysis ***Quantitation***, activate the button **Cycling A JOE** or **Cycling A Yellow**, **Show**. In the ***Select the sample page to use*** select ***Lsp***.
2. Cancel the automatic choice ***Threshold***.
3. Activate the ***Dynamic tube*** and **Slope Correct** buttons in the main window menu.
4. Activate the ***More Settings*/*Outlier Removal*** button and enter set **10 %**.
5. In ***CT Calculation*** menu set ***Threshold* = 0.03.**
6. In the ***Calc. Conc.*** column of the ***Quant. Results*** window the number of *Lactobacillus* spp. DNA copies in a PCR-sample will appear.
7. Note, that in the ***Standard Curve*** window, the R coefficient (coefficient of correlation) should be no less than 0.99. The **Efficiency** should fit in 0,85 – 1,08 range.

Calculation of concentration of *Gardnerella vaginalis* and *Lactobacillus* spp. DNA per ml and difference of logarithms of *L.sp. и G.v.* concentrations in test samples can be conveniently performed by computer applications.

Example of calculations performed in MS Excel:

Name	Calc Conc (copies/PCR)	Calc Conc (copies/PCR)	*G.vaginalis* (copies/ml)	*Lactobacillus* spp. (copies/ml)	lg(Lsp)-lg(Gv)
A	B	C	D = B*100	E = C*100	F = LOG(E)-LOG(D)
bv-	268	131336	2.7E+04	1.3E+07	2.7
bv-	271	133356	2.7E+04	1.3E+07	2.7
bv-	*89*	*98324*	*8.9E+03*	*9.8E+06*	*3.0*
bv+	21425	1006	2.1E+06	1.0E+05	-1.3
bv+	26288	1300	2.6E+06	1.3E+05	-1.3
bv+	20756	1026	2.1E+06	1.0E+05	-1.3
bv+	24761	1012	2.5E+06	1.0E+05	-1.4
k-		4			
GL1	204574	1127193			
GL2	2358	13004			
GL3	27	84			

AMPLIFICATION AND DATA ANALYSIS USING iCycler iQ and iCycler iQ5 (Bio-Rad, USA) INSTRUMENTS

For carrying out the amplification process it is recommended to use transparent PCR tubes (volume 0.2 ml) with domed or flat optically transparent caps (detection through the cap of the tube).

1. Switch on the instrument and the power supply unit of the optical module.

NOTE: The lamp should be warmed up for at least 15 min before starting the experiment.

2. Start the program iCycler iQ/iQ5.
3. Insert the tubes or strips into the reaction module of the thermocycler and program the instrument.

NOTE: Monitor the tubes. There must not be drops left on the walls of the tubes as falling drops during the amplification process may lead to the signal failure and complicate the results analysis. Don't turn the strips upside down while inserting them into the instrument.

Program the thermocycler according to the instruction manual given by the manufacturer of the instrument:

1. Click ***Create New*** in a ***Workshop*** module to enter a new amplification protocol. Set the amplification parameters (see tables 3, 4). Name a new protocol and save it.

Table 3

Amplification program for plate-type instruments

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

NOTE: Universal program**, AmpliSens-1 iQ**, 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.

Table 4

AmpliSens-1 amplification program for plate-type instruments

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycles*
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	
	72	15 s	–	

2. Create new ***Plate Setup***. Set the order of the tubes in the reaction chamber.
3. In the opened window set ***Unknown*** for all the clinical samples and "-" for all the negative controls. The calibrators in the **FAM** and **JOE/HEX** channels set as ***Standard*** and specify the concentration from the *Important Product Information Bulletin* enclosed to the kit. The ***Whole Plate Loading*** mustn't be activated. For all the samples and calibrators set the detection in **FAM** and **JOE/HEX** channels.
4. Set the reaction volume ***Sample Volume - 25 µl.***
5. Name the scheme of tubes' positions and save it.
6. Click ***Run.*** Tick ***Use Persistant Well Factors*** in the opened window, click ***Begin Run*** and save the experiment.

Data analysis:

1. Start the program and open the saved file by pressing the ***Data file*** button in the ***Workshop*** module. Switch to the ***Data Analysis*** mode.
2. Browse the data separately for each channel.
3. Check if the automatic choice of the threshold line for each channel is correct. Normally, the threshold line is to cross only with S-shaped curves describing the concentration of the signal detecting positive samples and controls. The threshold line is not to cross the base line. If it happens, it is necessary to raise the threshold level by pushing the ***Log View*** button and setting the threshold lines level (with the left mouse button) so that the

fluorescence curves should be of a linear character and not intersect with the curves of the negative samples. In the table of results (***Quant. Results***) one will be able to see the *Ct* values for the channel which is under analysis.

4. To analyse the results press the ***PCR Quant*** *(iQ5 iCycler)* button or activate the ***Results*** button (which is under the button with the fluorophores names) *(iQ5).*
5. It is recommended to calculate the data in Excel tables. For this the data should be copied or exported by clicking with right mouse button on the results grid. Select ***Export to Excel***. Save the file. If Microsoft Excel is installed the file will open automatically. (If the program is not installed data analysis should be continued on the computer where Microsoft Excel is installed.)

Example of calculations performed in MS Excel:

Name	Calc Conc (copies/PCR)	Calc Conc (copies/PCR)	*G.vaginalis* (copies/ml)	*Lactobacillus* sp. (copies/ml)	lg(*Lsp*)-lg(*Gv*)
A	B	C	D = B*100	E = C*100	F = LOG(E)-LOG(D)
bv-	268	131336	2.7E+04	1.3E+07	2.7
bv-	271	133356	2.7E+04	1.3E+07	2.7
bv-	89	98324	8.9E+03	9.8E+06	3.0
bv+	21425	1006	2.1E+06	1.0E+05	-1.3
bv+	26288	1300	2.6E+06	1.3E+05	-1.3
bv+	20756	1026	2.1E+06	1.0E+05	-1.3
bv+	24761	1012	2.5E+06	1.0E+05	-1.4
k-		4			
GL1	204574	1127193			
GL2	2358	13004			
GL3	27	84			

AMPLIFICATION AND DATA ANALYSIS USING Mx3000P and Mx3005P (Stratagene, USA) INSTRUMENTS

1. Switch on the instrument and start the Stratagene Mx3000P, Mx3005P program.
2. Choose ***Quantitative PCR (Multiple Standarts)*** in ***New Experiment Options*** window and tick the ***Turn lamp on for warm-up.***

NOTE: The lamp is to be be warmed up for at least 15 min before starting the experiment.

3. Insert the tubes into the instrument and close the lid.
4. In ***Options*** menu choose ***Optics Configuration*** and on the ***Dye Assignment*** tab set ***JOE*** in front of ***HEX/JOE filter set*** and set ***FAM*** in front of ***FAM filter set.***
5. Lock the fixing arm and the door of the instrument.
6. Choose ***Quantitative PCR (Multiple Standards)*** in ***New Experiment Options*** window and tick the ***Turn lamp on for warm-up.***
7. In ***Plate Setup*** menu set the parameters of fluorescence detection. To do this:
   - choose all cells in which the test tubes or strips are set (holding ***Ctrl*** and selecting the necessary region with the mouse).
   - mark all selected cells as ***Unknown*** in the ***Well type*** window. For the option ***Collect fluorescence data*** tick ***FAM*** and ***JOE***. After that click twice on each cell and enter the name for each test sample (the ***Well Information*** window). One can enter the signs of the samples during or after amplification returning to the ***Plate Setup*** menu.
8. On the ***Plate Setup*** tab set the parameters of fluorescence detection of the tubes. For this choose all cells in which the test tubes are set (holding ***Ctrl*** and selecting the necessary region with the mouse) in ***Well type*** drop-down menu choose the ***Unknown*** type and ***Collect fluorescence data*** field and tick ***FAM*** and ***JOE***; after that clicking twice on each box enter the signs of the tubes (into ***Well Information*** window), sign negative control as "-". Set calibrators as ***Standard*** in ***Well type*** window in the FAM and JOE channels and specify the concentration in the ***Standard quantity*** window. One can enter the signs of the samples during or after amplification returning to this tab.
9. Go to ***Thermal Profile Setup*** tab and set the amplification program. For this use one of the following methods:
   - **Using the template file to set the amplification program (is recommended)**
     Click the ***Import…*** button which is to the right of the depicted thermocycling profile. Select the fold containing the previous experimental file and open the fold. The needed thermocycling profile will appear in the ***Thermal Profile*** window.

– **Individual programming**

After setting all of the necessary parameters and values mark again all the cells in which the test tubes are set. Go to ***Thermal Profile Setup*** menu and set the amplification program (see tables 5, 6).

Table 5

Amplification program for plate-type instruments

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

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

Table 6

AmpliSens-1 Mx amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycles***
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	
	72	15 s	–	

For setting the parameter of the fluorescence signal detection at the set temperature it is necessary to select the ***All points*** option for the ***Data collection marker for dragging*** parameter and drag it with a mouse from the right part of the box to the field with the necessary temperature.

10. Start the amplification by clicking the ***Run*** and ***Start*** buttons, then name the experiment.

Data analysis:

The obtained data are interpreted by the software of the Mx3000P and Mx3005P instruments on the basis of presence the crossing of fluorescence curve with the threshold line set at the specific level (it correspond to the presence of threshold cycle (*Ct*) value in the corresponding column of the results grid).

1. In the Mx3000P program Select ***Analysis*** by clicking the corresponding button of the tool bar.

2. The ***Analysis Selection/Setup*** tab will open. Make sure that all the test samples are active (the cells corresponding to the samples should be of a different colour). Otherwise select all the test samples by holding down the ***Ctrl*** button and selecting the needed range with the mouse.
3. Select the ***Results*** tab.
4. For each channel check the correctness of automatic choice of the threshold line. Normally the threshold line must cross only S-shaped curves of signal accumulation of positive samples and controls and mustn't cross the base line. If it is not correct the level of the base line should be raised. For this activate the FAM channel and switch off other channels on the bottom of ***Dyes shown*** panel. Check the position of the threshold and change it if it's necessary. After that consequently selecting the next channel and turning off the previous channel check the position of the thresholds.

The curves of the signal accumulation are shown by the instrument in the linear form by default. In order to change the form of the curves and make them logarithmical double click the left mouse button near one of the coordinate axes (X or Y) in the ***Graph properties*** window for Y axis (***Y axis***) and put the tick mark against the ***Log*** point in the ***Scale*** field.

5. Activate two fluorescent channels (***FAM, JOE*** are to be activated in ***Dyes Shown*** field at the bottom of the window).
6. Select ***Text Report*** in the ***Area to analyze*** field. Make sure that all the data are sorted according to the dye name (***Dye*** column). For this click once on the name of the column.
7. Select the ***File*** menu, then ***Export Text Report*** and next ***Export Text Report to Excel.*** Microsoft Excel window will open.
8. It is recommended to make the calculations with the help of electronic worksheet, for example Excel. For this one should copy data to the electronic worksheet or export data by clicking with right mouse button on the appeared results grid. In the drop-down menu select ***Export to Excel***. Save the file. If Microsoft Excel is installed on the computer the file will open automatically. If the program is not installed on the computer the following analysis should be carried out on the computer with the installed Microsoft Excel.

Example of calculations performed in MS Excel:

Name	Calc Conc (copies/PCR)	Calc Conc (copies/PCR)	*G.vaginalis* (copies/ml)	*Lactobacillus* spp. (copies/ml)	lg(Lsp)-lg(Gv)
A	B	C	D = B*100	E = C*100	F = LOG(E)-LOG(D)
bv-	268	131336	2.7E+04	1.3E+07	2.7
bv-	271	133356	2.7E+04	1.3E+07	2.7
bv-	89	98324	8.9E+03	9.8E+06	3.0
bv+	21425	1006	2.1E+06	1.0E+05	-1.3
bv+	26288	1300	2.6E+06	1.3E+05	-1.3
bv+	20756	1026	2.1E+06	1.0E+05	-1.3
bv+	24761	1012	2.5E+06	1.0E+05	-1.4
k-		4			
GL1	204574	1127193			
GL2	2358	13004			
GL3	27	84			

AMPLIFICATION AND DATA ANALYSIS USING CFX96 (Bio-Rad, USA)

When carrying out the amplification it is recommended to use thin-walled PCR tubes (0.2 ml) with domed or flat optically transparent caps or tubes (0.2 ml) with transparent caps from the eight-pieces-strips (e.g. Axygen, USA) (detection through the cap of the tube).

1. Turn on the instrument and start the ***Bio-Rad CFX Manager*** program.
2. In the start window select the ***Create a new Run*** button. Set amplification program (see Table 7, 8).

Table 7

Amplification program for plate-type instruments

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

NOTE: Universal program**, AmpliSens-1**, can be used as well (see table 7). The program allows conducting any combination of tests (for example, for detection of DNA of sexually transmitted infections) in a single run.

Table 7

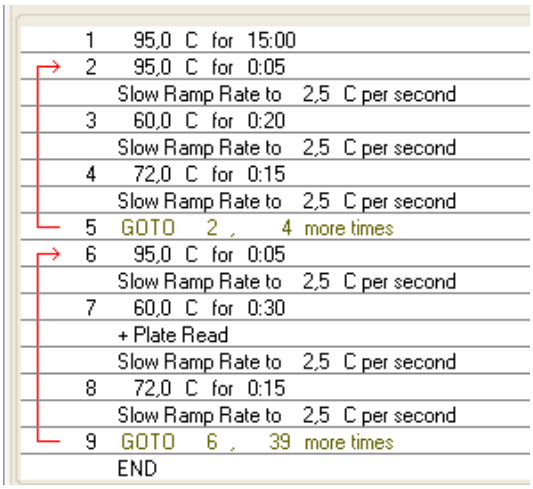
AmpliSens-1 amplification program for plate-type instruments

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

To do this, select or create the program in the ***Experiment Setup*** tab in the ***Protocol Editor*** window. Set the ***Sample Volume*** as ***30 µl***.

NOTE! Set ***Ramp Rate* 2,5 °C/s** by clicking the ***Step Options*** button for each step of cycling (see figure 1).

Figure 1



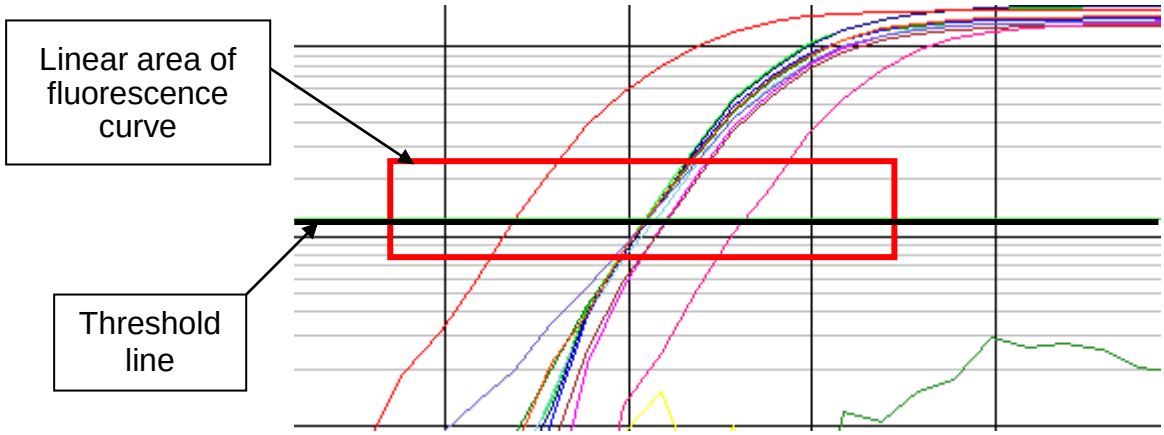
3. In the ***Plate*** tab click set the tube order in the reaction chamber and fluorescence detection in the FAM and HEX channels for all samples. For this tick required fluorophores by the ***Select Fluorophores…*** button. Set the type ***Unknown*** opposite all the test samples and negative controls. Set the type ***Standard*** opposite calibrators and specify their concentrations according to *the Important Product Information Bulletin* in the ***Concentration*** field. Save the plate scheme, click the ***OK*** button.
4. Open the instrument lid by the ***Open Lid*** button. Place the reaction tubes in the wells of the instrument in accordance with the entered plate setup. Close the lid by the ***Close Lid*** button.
5. Start the selected amplification program with the set plate scheme by clicking ***Start Run*** button.
6. Proceed to the analysis of results after the end of the run.

Data analysis

Obtained data are interpreted by the real-time PCR instrument software by the crossing (or not crossing) of a fluorescence curve with the threshold line set at the specific level (that corresponds to the presence (or absent) of *Ct* (*Cq*) value in the results grid).

1. Start the program and open the saved file. To do this, Select ***File*** in the menu, then ***Open*** and ***Data file*** and select the file.
2. Browse the data separately for each channel, excluding other channels (clear a tick box with cannel designation under the main window with ***Amplification*** curves).
3. Set the threshold line for each cannel so that the threshold line cross the fluorescence curves at the area of typical exponential growth of fluorescence and do not cross the base line. If automatic choice of threshold level do not corresponds to this requirement it is necessary to raise the threshold level by clicking the ***Log View*** button and setting the threshold lines level (with the left mouse button) so that the fluorescence curves of DNA-calibrators should be of a linear character and stepping up from base line fluctuation of the negative control samples (see figure 2).

Figure 2



4. The threshold cycles (*Cq*) values for the analyzed channel will appear in the results grid.
5. *Cq* values for the negative control samples are to be absent in two channels.
6. *Cq* values for the calibrators are to be determined in two channels.
7. Amplification effectiveness ***E*** in the ***Standard Curve*** window for each channel is to be in the range 80-120 %.
8. It is recommended to make the calculations with the help of electronic worksheet, for example Excel. For this one should copy data to the electronic worksheet end enter the formula as follows:

Example of calculations performed in MS Excel:

Name	Calc Conc (copies/PCR)	Calc Conc (copies/PCR)	*G.vaginalis* (copies/ml)	*Lactobacillus sp.* (copies /ml)	lg(*Lsp*)-lg(*Gv*)
A	B	C	D = B*100	E = C*100	F = LOG(E)-LOG(D)
bv-	268	131336	2.7E+04	1.3E+07	2.7
bv-	271	133356	2.7E+04	1.3E+07	2.7
bv-	89	98324	8.9E+03	9.8E+06	3.0
bv+	21425	1006	2.1E+06	1.0E+05	-1.3
bv+	26288	1300	2.6E+06	1.3E+05	-1.3
bv+	20756	1026	2.1E+06	1.0E+05	-1.3
bv+	24761	1012	2.5E+06	1.0E+05	-1.4
k-		4			
GL1	204574	1127193			
GL2	2358	13004			
GL3	27	84			

List of Changes Made in the Guidelines

VER	Location of changes	Essence of changes
05.11.14 ME	Text	Text was corrected in accordance with the template
	Intended use	The Rotor-Gene Q (QIAGEN, Germany), iCycler iQ (Bio-Rad, USA), CFX96 (Bio-Rad, USA) instruments was added
	Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia) AND Rotor-Gene Q (QIAGEN, Germany) instruments, Amplification and data analysis with the use of iCycler iQ and iCycler iQ5 (Bio-Rad, USA) instruments, Amplification and data analysis with the use of Mx3000P and Mx3005P (Stratagene, USA) instruments	The amplification programs was added
	Amplification and data analysis using CFX96 (Bio-Rad, USA)	The chapter was added
14.05.15 PM	Text	Clinical material was changed to biological
13.04.18 PM	Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia) AND Rotor-Gene Q (QIAGEN, Germany) instruments	The ***Efficiency*** value range was changed
29.12.20 MA	Through the text	The symbol  was changed to NOTE:
	Cover page	The phrase “Not for use in the Russian Federation” was added
02.06.21 KK	Cover page	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