IVD

For Professional Use Only

GUIDELINES

to **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit for qualitative detection of *Borrelia burgdorferi sensu lato (B. burgdorferi sensu stricto, B. afzelii, B. garinii)* 16S rRNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection

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Not for use in the Russian Federation

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INTENDED USE

The guidelines describe the procedure of using **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit for qualitative detection of *Borrelia burgdorferi sensu lato (B. burgdorferi sensu stricto, B. afzelii, B. garinii)* 16S rRNA in the biological material (ticks) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection using the following instruments:

- Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia).

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

When working with Rotor-Gene 3000 one should use the Rotor-Gene version 6 software. When working with the Rotor-Gene 6000 one should use the the Rotor-Gene 6000 versions 1.7 (build 67) software or higher.

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

Carry out sample pretreatment and reaction mixture preparation stages according to the PCR kit instruction manual.

Programming the thermocycler

1. Turn on the instrument, run the Rotor-Gene software.
2. Insert the tubes into the rotor of the Rotor-Gene 3000/6000 instrument (the rotor wells are numbered, the numbers are used for the further programming of the samples' order in the thermocycler). Program the instrument.

NOTE: Well No. 1 must be filled with any test tube.

3. Set the amplification program.

Amplification program of *Borrelia burgdorferi sensu lato* cDNA

Step	Temperature	Time	Fluorescence detection	Cycles
1	95 °C	15 min	-	1
2	95 °C	15 s	-	10
	63 °C	50 s	-	
	72 °C	20 s	-	
3	95 °C	15 s	-	40
	58 °C	50 s	FAM/Green, JOE/Yellow	
	72 °C	20 s	-	

4. Set the following calibration parameters (activate ***Calibrate/Gain Optimisation*** button in the ***New run wizard***):

- activate the ***Calibrate Acquiring***/***Optimise Acquiring*** button for the FAM/Green and JOE/Yellow channels.
- activate the ***Perform Calibration. Before 1st Acquisition/Perform Optimisation Before 1st Acquisition*** option for the FAM/Green and JOE/Yellow channels.
- set ***Min Reading*** **5Fl**, ***Max Reading*** **10Fl** for FAM/Green channel for the first tube and ***Min Reading*** **5Fl, *Max Reading*** **10Fl** for JOE/Yellow channel (activate select *Edit*, *Auto gain calibration channel settings* window).

5. Select the ***Start run*** button to run the amplification program and name the experiment.
6. During the thermocycler work or after it set the positions of the test samples, positive and negative controls of extraction and RT-PCR.

   Enter the data into the grid of the samples (it opens automatically after the amplification has been started). Enter the names/numbers of the test samples in the ***Name*** column. Define the Negative control of amplification as NCA, the Positive control of amplification as C+. Set the type ***Unknown*** opposite all the test samples, the type ***Positive control*** – for the positive controls, the type ***Negative control*** – for the Negative control of extraction, the type ***NTC*** – for the Negative control of amplification. Set the type ***None*** for the cells matching with the corresponding empty tubes.

Data analysis

Data analysis of the *Borrelia burgdorferi sensu lato* cDNA amplification (FAM/Green channel)

1. Activate the button ***Analysis*** in the menu, select the mode of the analysis ***Quantitation***, activate the buttons ***Cycling A. FAM/Cycling A. Green***, ***Show***.
2. Cancel automatic choice of threshold level ***Threshold***.
3. Activate the ***Dynamic tube*** and ***Slope Correct*** buttons in the menu of main window (***Quantitation analysis***).
4. In ***CT Calculation*** menu (in the right part of the window) indicate the threshold line level ***Threshold*** **= 0.03,** set ***Quantitation settings*** **– 5 %.**
5. In the results grid (the ***Quantitation Results*** window) one will be able to see the *Ct* values.
6. No *Ct* values should be detected for the Negative Control of extraction (C–).
7. *Ct* value for the Positive Control of extraction (PCE) – Positive Control *Borrelia burgdorferi sensu lato*-rec– should not exceed the value specified in the *Important*

Product Information Bulletin.

8. *Ct* value for the Positive Control of RT-PCR (C+) – Positive Control cDNA *Borrelia burgdorferi sensu lato*-rec (C+$_{B.\ burgdorferi\ sl}$) – should not exceed the value specified in the *Important Product Information Bulletin*.
9. The test samples are considered positive if the *Ct* value is less than the boundary value specified in the Important Product Information Bulletin. If the *Ct* value exceed the boundary value in FAM/Green channel and *Ct* value exceed the boundary value in the JOE/Yellow channel, then the analysis result is invalid. The PCR analysis should be repeated starting from the RNA extraction stage

Data analysis of Internal Control cDNA amplification (JOE/Yellow channel)

1. Activate the button ***Analysis*** in the menu, select the mode of the analysis ***Quantitation***, activate the buttons ***Cycling A. JOE/Cycling A. Yellow***, ***Show***.
2. Cancel automatic choice of threshold level ***Threshold***.
3. Activate the ***Dynamic tube*** and ***Slope Correct*** buttons in the main window menu (***Quantitation analysis***).
4. In ***CT Calculation*** menu (in the right part of the window) indicate the threshold line level ***Threshold* = 0.03,** set ***Quantitation settings* – 10 %**.
5. The *Ct* (Threshold cycle) values for **Internal Control STI-87-rec** must be shown in the results grid for all clinical samples and Positive and Negative Controls of extraction (PCE and C–).
6. No *Ct* values should be detected for the Negative Control of amplification (NCA), DNA-buffer.
7. The samples are valid if the *Ct* values do not exceed the boundary values value specified in the *Important Product Information Bulletin.*

NOTE: If the fluorescence curves in the JOE/Yellow channel does not correspond to the exponential growth, then select *NTC threshold* value in the range from 5 to 15 %

List of Changes Made in the Guidelines

VER	Location of changes	Essence of changes
18.04.13 FN	Footer	The reference number **REF** R-B37(RG)-CE-B was deleted
01.04.14 SA	Cover page	Address of European representative was added
01.06.15 ME	Text	Corrections in accordance with the template
	Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia)	The phrase “If the fluorescence curves in the JOE/Yellow channel does not correspond to the exponential growth, then select *NTC threshold* value in the range from 5 to 15 %” was added for data analysis in the JOE/Yellow channel
29.12.20 KK	Cover page	The phrase “Not for use in the Russian Federation” was added
	Through the text	The symbol  was changed to NOTE:
17.03.21 VA	Front page	The name, address and contact information for Authorized representative in the European Community was changed