GUIDELINES

to AmpliSens® *MTC*-diff-FRT PCR kit

for differentiation of mycobacteria tuberculosis (*M.tuberculosis*, *M.bovis* and *M.bovis* BCG) in the clinical material and microorganism cultures by the polymerase chain reaction (PCR) with real-time fluorescence hybridization detection

EC REP

Ecoli Dx, s.r.o., Purkyňova 74/2
110 00 Praha 1, Czech Republic
Tel.: +420 325 209 912
Cell: +420 739 802 523

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

Not for use in the Russian Federation

TABLE OF CONTENTS

INTENDED USE

The guidelines describe the procedure of using **AmpliSens® *MTC*-diff-FRT** PCR kit for differentiation of mycobacteria tuberculosis (*M.tuberculosis*, *M.bovis* and *M.bovis* BCG) in the clinical material and microorganism cultures 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);
- Rotor-Gene Q (QIAGEN, Germany);
- iCycler iQ, iQ5 (Bio-Rad, USA);
- Smart Cycler II (Cepheid, USA)
- CFX96, CFX96 Touch (Bio-Rad, USA).

Table 1

Compliance of the fluorophore channels with the detection channels names.

Fluorophore channel	Detection channels names for different instrument models[1]
FAM fluorophore channel	FAM/Green
JOE fluorophore channel	JOE/HEX/R6G/Yellow/Cy3
ROX fluorophore channel	ROX/Orange/TxR
Cy5 fluorophore channel	Cy5/Red

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, 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.

Carry out the sample pre-treatment and reaction mixture preparation stages according to the PCR kit *Instruction Manual*. When carrying out the amplification it is recommended to use thin-walled PCR tubes (0.2 ml) with flat caps (e.g. Axygen, USA), or Rotor-Gene PCR tubes (0.1 ml) with caps from the four-pieces-strips (e.g. Corbett Research, Australia; QIAGEN, Germany) (detection through the bottom of the tube).

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).

NOTE: The rotor's **well №1** must be filled with any sample tube from this experiment except for an empty one. If the sample tubes loaded into the rotor are being analyzed by different reagents kits, then **well №1** must be filled with a sample

---

[1] Detection channel name for the instrument in use is given in the respective section of the Guidelines for this reagents kit.

of highest fluorophore level. For example, in a simultaneous rotor loading of tubes tested for presence of *Mycobacterium tuberculosis complex* for quantitative analysis or differentiation, **well №1** must be filled with tubes containing quantitative detection reagents or *Mycobacterium tuberculosis complex* differentiation reagents.

Programming the thermocycler

1. Switch the instrument on and press **New** in the software main menu.

2. In the newly opened window, **New Run**, select the template **Advanced** and choose **Dual Labelled Probe/Hydrolysis probes**. Press the **New** button.

3. In the newly opened window select rotor for 36 wells, **36-Well Rotor**, and outline that tubes with domed lids are not being used (**No Domed Tubes** (Rotor-Gene 3000)) and that the locking ring is adjusted (**Locking Ring Attached** (Rotor-Gene 6000 and Rotor-Gene Q)). Press the **Next** button.

4. In the newly opened window set the name of the operator and choose the reaction mix volume: **Reaction volume** – **25 µl**. For Rotor-Gene 6000 or Rotor-Gene Q tick the box next to **15 µl oil layer volume**. Press the **Next** button.

5. In the newly opened window set the temperature profile for the experiment. To do this, press the **Edit profile** button and set the amplification parameters to **95-65-72 MTC**.

95-65-72 MTC amplification program

Step	Temperature, °C	Time	Fluorescence detection	Number of cycles
Hold	95	15 min	–	1
Cycling	95	15 s	–	5
	65	30 s	–	
	72	15 s	–	
Cycling 2	95	15 s	–	40
	65	30 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 s	–	

6. Twice press the **OK** button.

7. Set the automatic calibration in order to select the **gain** parameter. To do this, in the **New Run Wizard** press the **Calibrate/Gain Optimisation**. In the newly opened window, **Auto Gain Calibrator Setup**, press **Calibrate Acquiring/Optimise** button, and tick the box in the **Perform Calibration Before 1st Acquisition/Perform Optimisation Before 1st Acquisition** line. For all channels set the **Min reading** to **5 Fl** and the **Max Reading** at **10 Fl**. Press the **Close** button to close this window.

8. Activate the **Next** button, and start amplification by pressing **Start run**.

9. Name the experiment and save it on the disc (this file will automatically save the results of the experiment).

10. Insert the data into the samples table (it will open automatically after amplification starts). In the **Name** column insert the names/numbers of the tested clinical samples. The negative control of PCR must be designated as **NCA** and the positive control as **C+**. For empty wells select the **None** type.

NOTE: Samples indicated as **None** will not be analysed.

<u>**Data analysis:**</u>

Results analysis for the amplification in the FAM/Green channel:

1. Activate the button **Analysis** in the menu, select the mode of the analysis **Quantitation**, and activate the buttons **Cycling A. FAM /Cycling A. Green**, press the **Show** button.

2. Discard the popup window for automatic choice of the **Threshold** line level.

3. In the main window menu (**Quantitation analysis**) activate the buttons **Dynamic tube** and **Slope Correct**.

4. Scale the graphic curves by pressing the **Auto-Scale** button at the bottom of the **Quantitation Analysis – Cycling A. Green** window.

5. In the **CT Calculation** menu (in the right part of the window) indicate the threshold line level **0.03** in the **Threshold** box.

6. Choose the parameter **More settings/Outlier Removal** and set **10 %** for the value of negative samples threshold (**NTC/Threshold**).

7. In the results grid (the **Quant. Results – Cycling A. Green** window) one will be able to see the **Ct** values.

Results analysis for the amplification in the JOE/Yellow channel:

1. Activate the **Analysis** button, select the **Quantitation** analysis, and activate the **Cycling A. JOE/Cycling A. Yellow**, press **Show** button.

2. Cancel the automatic choice of the threshold line level (**Threshold**)

3. In the main window menu (**Quantitation analysis**) activate the **Dynamic tube** and the **Slope Correct** buttons.

4. Scale the graphic curves by pressing the **Auto-Scale** button at the bottom of the **Quantitation Analysis – Cycling A. Yellow** window.

5. In the **CT Calculation** menu (in the right part of the window) indicate the threshold line level **0.05** in the **Threshold** box.

6. Choose the parameter **More settings/Outlier Removal** and set **10 %** for the value of negative samples threshold (**NTC/Threshold**).

7. In the results grid (the **Quant. Results – Cycling A. JOE/Quant. Results – Cycling A. Yellow** window) one will be able to see the **Ct** values.

Results analysis for the amplification in the ROX/Orange channel:

1. Activate the **Analysis** button, select the **Quantitation** analysis, and activate the **Cycling A. ROX/Cycling A. Orange**, press **Show** button.

2. Discard the popup window for automatic selection of the **Threshold** line level.

3. In the main window menu (**Quantitation analysis**) activate the **Dynamic tube** and the **Slope Correct** buttons.

4. Scale the graphic curves by pressing the **Auto-Scale** button at the bottom of the **Quantitation Analysis – Cycling A. Orange** window.

5. In the **CT Calculation** menu (in the right part of the window) indicate the threshold line level **0.05** in the **Threshold** box.

6. Choose the parameter **More settings/Outlier Removal** and set **10 %** for the value of negative samples threshold (**NTC/Threshold**).

7. In the results grid (the **Quant. Results – Cycling A. ROX/Quant. Results – Cycling A. Orange** window) one will be able to see the **Ct** values.

Results analysis for the amplification in the Cy5/Red channel:

1. Activate the **Analysis** button, select the **Quantitation** analysis, and activate the **Cycling A. Cy5/Cycling A. Red**, press **Show** button.

2. Discard the popup window for automatic selection of the **Threshold** line level.

3. In the main window menu (**Quantitation analysis**) activate the **Dynamic tube** and the **Slope Correct** buttons.

4. Scale the graphic curves by pressing the **Auto-Scale** button at the bottom of the **Quantitation Analysis – Cycling A. Red** window.

5. In the **CT Calculation** menu (in the right part of the window) indicate the threshold line level **0.05** in the **Threshold** box.

6. Choose the parameter **More settings/Outlier Removal** and set **30 %** for the value of negative samples threshold (**NTC/Threshold**).

7. In the results grid (the **Quant. Results – Cycling A. Cy5/Quant. Results – Cycling A. Red** window) one will be able to see the **Ct** values.

NOTE: Results interpretation for controls and for clinical material must be carried out in compliance with the *Instruction Manual* and *Important Product Information Bulletin*.

AMPLIFICATION AND DATA ANALYSIS USING iCycler iQ5 (Bio-Rad, USA) INSTRUMENT

Carry out the sample pretreatment and reaction mixture preparation stages according to the PCR kit *Instruction Manual*. When carrying out the amplification it is recommended to use thin-walled PCR tubes (0.2 ml) with domed or optically transparent flat 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).

Program the thermocycler:

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

NOTE: The lamp should be warmed up for at least 30 min before starting the experiment.

2. Start the program **iCycler iQ5**.

3. Insert the tubes, strips or plate into the reaction module of the thermocycler and program the instrument.

NOTE: Monitor the tubes, so that no drops are left on the walls of the tubes, if the drops fall during the amplification process it may lead to the signal failure and complicate the results analysis. Don't turn the strips/plates upside down while inserting them into the instrument.

4. Set the plate setup (set the order of the tubes in the reaction chamber along with the detection of fluorescent signal). To do this click the **Create New** or **Edit** buttons in the **Selected Plate Setup** window of the **Workshop** module. One can edit the plate setup in the **Whole Plate loading** mode. In the Select and load option set the fluorescent signal detection in all tubes in channels FAM, JOE/HEX, ROX and Cy5. Set the reaction volume (**Sample Volume**) as **25 μl**, set the caps type (**Seal Type**) as **Domed Cap**, and the tubes type (**Vessel Type**) as **Tubes**. Save the set plate setup by clicking the **Save&Exit Plate Editing** button.

5. Set the **95-65-72 MTC** amplification program.

95-65-72 MTC amplification program

Step	Temperature, °C	Time	Fluorescence detection	Number of cycles
Hold	95	15 min	–	1
Cycling	95	15 s	–	5
	65	30 s	–	
	72	15 s	–	
Cycling 2	95	15 s	–	40
	65	30 s	FAM, JOE/HEX, ROX, Cy5	
	72	15 s	–	

To create a protocol in **Selected Editing** window **Workshop** module, press **Create New** or **Edit** button. Set amplification parameters and save the protocol by pressing **Save&Exit**

Protocol Editing. For future loading one may choose the file with this program in ***Protocol*** block (by default the protocol files are saved to ***Users*** folder).

6. Launch the chosen program with the set plate setup. Before launching the program check that ***Selected protocol*** and ***Selected Plate Setup*** reflect the setup you have chosen. To launch press Run button. Select ***Use Persistent Well Factors*** for factor evaluation (will be set by default). Press ***Begin Run*** button. Name your experiment (this file will automatically save the results for this experiment) and press ***OK***.

<u>Data analysis:</u>

1. After amplification has finished select the ***Data analysis*** mode. The data is given for each channel separately.

2. In the module window select the data for the analysed channel. Herewith the data analysis mode must be ***PCR Base Line Subtracted Curve Fit*** (selected by default). Set the threshold line level respective to 15 % of the maximum fluorescence level, obtained for **Positive Control DNA *MTC*-diff / STI (C+ *MTC*-diff / STI)** in the last cycle of amplification for all channels. The fluorescence level of a sample is believed to be equal to the closest upper division of the scale, that denotes the numerical value. Herein it is neccessary that the fluorescence curve for **Positive Control DNA *MTC*-diff / STI (C+ *MTC*-diff / STI)** resembles the characteristic sigmoid shape. It is possible to use the automatically set threshold line level, given that it falls within the defined region. In order to highlight the sample curve use the ***Display Wells*** button or place your coursor on the curve for that sample and **double click** on it.

3. In order to change the levele of the threshold line, drag it with your left mouse button, or select ***Baseline Threshold*** menu (in the dropdown menu, when you click the right mouse button on the fluorescence graphs), then choose the ***User Defined*** option and enter the correct value in the ***Threshold Position*** field. To see results table click ***Results*** button.

NOTE: Results interpretation for controls and for clinical material must be carried out in compliance with the *Instruction Manual* and *Important Product Information Bulletin*.

AMPLIFICATION AND DATA ANALYSIS USING SmartCycler (Cepheid, USA) INSTRUMENT

1. Before loading the thermocycler place the reaction mix into the lower thermocycling part of the tube. To do this, one needs to place the tubes into the rotor of specific Mini-Spin centrifuge (Cepheid, USA) and switch it on for 5-7 s.

2. Place the tubes into the thermocycler wells and close the well lids.

Program the thermocycler:

1. Open the software for SmartCycler.

2. In the main menu select **Define Protocols**. In a newly opened window select the **New Protocol** button in the bottom left corner and name the protocol **95-65-72 MTC**, program the instrument to run **95-65-72 MTC** setup for SmartCycler (Cepheid, USA).

95-65-72 MTC amplification program for SmartCycler (Cepheid, USA)

Temperature, °C	Time	Number of cycles
95	900 s	1
95	20 s	
65	50 s	45
72	20 s	

3. Press **Save Protocol** button at the bottom of the window.

4. Select **Create Run** in the main menu. Press **Dye set** button in the central left part of the window and select the **FCTC25** dye combination.

5. Click the **Add/Remove Sites** button at the centre of the window. In the newly opened window select the required protocol (program) and select the analyzed wells. Click **OK**.

6. Click **Start Run** button at the bottom of the window to run the program. In the popup dialogue window enter the file name you want the results to be stored in.

7. All settings of this experiment are given in the table at the top of the window. The table contains a **Sample Type** column that indicates each sample as **UNKN** (unknown) by default. In the **Sample ID** column name each sample.

<u>**Data analysis**</u>

1. Select **Analysis settings** in the menu. Set the threshold line level to **30** for each channel, **FAM, Cy3, Texas Red and Cy5**.

2. In the **Results Table** window *Ct* values will appear for **FAM, Cy3, Texas Red** and **Cy5** channels.

The results can be interpreted using the automatic evaluation, reflected in the results table (***Results***), or by visual analysis of the fluorescence curves for **FAM, Cy3, Texas Red** and **Cy5** channels**.**

Results for controls

PCR analysis result is considered reliable if correct results are obtained for both positive and negative controls of amplification, according to the Table 2.

Table 2

Results for controls of amplification using SmartCycler

Control	Stage for control	Result in channel			
		FAM	**Cy3**	**Texas Red**	**Cy5**
C+	PCR	POS	POS	POS	POS
NCA	PCR	NEG	NEG	NEG	NEG

Results for clinical material and microorganism cultures

Table 3

Results interpretation for analysed samples using SmartCycler

DNA of mycobacteria strain	FAM	Cy3	Texas Red	Cy5	Result	Result interpretation
M.tuberculosis	POS	POS / NEG	POS / NEG	POS / NEG	Detected	*M.tuberculosis* DNA (human strain) is detected
	NEG	POS / NEG	POS / NEG	POS	Not detected	*M.tuberculosis* DNA (human strain) is not detected
	NEG	POS / NEG	POS / NEG	NEG	Invalid	*M.tuberculosis* DNA (human strain) – detection result is invalid. Repeat sampling
M.bovis	POS / NEG	POS	POS / NEG	POS / NEG	Detected	*M.bovis* DNA (bovine strain) is detected.
	POS / NEG	NEG	POS / NEG	POS	Not detected	*M.bovis* DNA (bovine strain) is not detected
	POS / NEG	NEG	POS / NEG	NEG	Invalid	*M.bovis* DNA (bovine strain) –detection is invalid. Repeat sampling

DNA of mycobacteria strain	FAM	Cy3	Texas Red	Cy5	Result	Result interpretation
M.bovis BCG	POS / NEG	POS / NEG	POS	POS / NEG	Detected	*M.bovis* BCG DNA(vaccine strain) is detected
	POS / NEG	POS / NEG	NEG	POS	Not detected	ДНК *M.bovis* BCG (вакцинный штамм) is not detected
	POS / NEG	POS / NEG	NEG	NEG	Invalid	*M.bovis* BCG DNA (vaccine strain) – detection result is invalid. Repeat sampling

Interpretation of results

– *M.tuberculosis* DNA is **detected** in a sample if the results table for the tested sample in the FAM channel displays a **POS** result ($Ct_{FAM} \neq 0$) in **Std/Res FAM** column. Herein the fluorescence curve for the tested sample must intercept the threshold line at the area of characteristic exponential growth of fluorescence.

– *M.bovis* DNA is **detected** in a sample if the results table for the tested sample displays a **POS** result ($Ct_{Cy3} \neq 0$) in **Std/Res Cy3** column. Herein the fluorescence curve for the tested sample must intercept the threshold line at the area of characteristic exponential growth of fluorescence.

– *M.bovis* **BCG** DNA is **detected** in a sample if the results table for the tested sample displays a **POS** result ($Ct_{TexasRed} \neq 0$) in **Std/Res Texas Red** column. Herein the fluorescence curve for the tested sample must intercept the threshold line at the area of characteristic exponential growth of fluorescence. Due to the fact that the vaccine strain *M.bovis* **BCG** is part of the bovine strain MBT (*M.bovis*), the results table may also display a **POS** result ($Ct_{Cy3} \neq 0$) in **Std/Res Cy3** column.

– *M.tuberculosis* DNA, *M.bovis* DNA and *M.bovis* **BCG** DNA are **not detected** in a sample if its fluorescence curve for the **FAM**, **Cy3** and **Texas Red** channels does not intercept the threshold line (the results table displays **NEG** results ($Ct_{FAM}=0$, $Ct_{Cy3}=0$, $Ct_{TexasRed}=0$) in respective columns, therewith **Std/Res Cy5** displeys a **POS** result ($Ct_{Cy5} \neq 0$) or **NEG** result ($Ct_{Cy5}=0$).

– The validity of results is interpreted independently. If the results table columns **Std/Res FAM** and **Std/Res Cy5** or **Std/Res Cy3** and **Std/Res Cy5** or **Std/Res Texas Red** and **Std/Res Cy5** display **NEG** results ($Ct_{FAM}=0$, $Ct_{Cy3}=0$, $Ct_{TexasRed}=0$, $Ct_{Cy5}=0$, respectively), then the amplification of that sample needs to be repeated, in case of

obtaining an analogous result it is necessary to repeat the sample analysis starting from the DNA/RNA extraction step. If a valid result is not obtained, then differentiation result of the respective **MBT** strain is interpreted as invalid, therewith recommended repetition of the sampling step.

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

Carry out the sample pretreatment and reaction mixture preparation stages according to the PCR kit *Instruction Manual*. When carrying out the amplification it is recommended to use thin-walled PCR tubes (0.2 ml) with domed or optically transparent flat 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).

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 instrument according to the *Instruction Manual* provided by the manufacturer.

1. Turn on the instrument and start the ***Bio-Rad CFX Manager*** program.

2. Select ***Create a new Run*** in the ***Startup Wizard*** start screen (or select ***New*** and then ***Run…/Experiment…*** in the ***File/Experiment*** menu).

3. In the ***Run Setup*** window, select ***Protocol*** and click the ***Create new…*** button. Set amplification parameters (time, temperature, cycles, and fluorescence acquiring cycle) in the opened ***Protocol Editor – New*** window (see Table 4). Set ***Sample Volume – 25 µl.***

Table 4

95-65-72 MTC amplification program

Step	Temperature, °C	Time	Fluorescence detection	Number of cycles
1	95	15 min	–	1
2	95	15 s	–	5
	65	30 s	–	
	72	15 s	–	
3	95	15 s	–	40
	65	30 s	FAM, HEX, ROX, Cy5	
	72	15 s	–	

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

Figure 1

```
1    95,0  C  for  15:00
2    95,0  C  for  0:15
     Slow Ramp Rate to  2,5  C per second
3    65,0  C  for  0:30
     Slow Ramp Rate to  2,5  C per second
4    72,0  C  for  0:15
     Slow Ramp Rate to  2,5  C per second
5    GOTO  2 ,      4  more times
6    95,0  C  for  0:15
     Slow Ramp Rate to  2,5  C per second
7    65,0  C  for  0:30
     + Plate Read
     Slow Ramp Rate to  2,5  C per second
8    72,0  C  for  0:15
     Slow Ramp Rate to  2,5  C per second
9    GOTO  6 ,     39  more times
     END
```

4. In the *Protocol Editor New* window select *File*, then *Save As*, and name the protocol. This protocol can be used for further runs by clicking the *Select Existing…* button in the *Protocol* tab.

   When the required program is entered or edited, click *OK* at the bottom of the window.

5. Set the plate setup. In the *Plate* tab click the *Create new…* button. Set the tube order in the opened *Plate Editor – New* window. In the *Sample type* menu select *Unknown*; click the *Select Fluorophores…* button and indicate in the *Selected* column the required fluorophores with a checkmark; click *OK*; then indicate with a checkmark the fluorescence signal acquiring for the selected wells in the required channels. Define sample names in the *Sample name* window (the parameter *Load* should be marked with a checkmark).

6. In the *Plate Editor New* window select *File*, then *Save As*, and name the plate. When the required plate is entered or edited, click *OK* at the bottom of the window.

7. Select the *Start Run* inlay*.* Click the *Open Lid* button to open the instrument. Place the reaction tubes in the wells of the instrument in accordance with the entered plate setup. Click the *Close Lid* button to close the instrument.

8. To start the program run activate the *Start Run* button in the *Start Run* tab then save the file of the experiment.

9. 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 according to the crossing (or not-crossing) of the S-shaped fluorescence curve with the threshold line (set in the middle of the linear fragment of fluorescence growth of the positive control in the log

scale) and shown as the presence (or absence) of the *Ct* (threshold cycle) value in the results grid.

1. Start the program, then in the **File** menu activate the **Open** and **Data file** buttons to select required file.
2. The fluorescence curves, plate setup, and results grid with *Ct* values are displayed in the **Data Analysis** window of the **Quantification** tab

Variant 1

For each channel at a time set the threshold line (drag it with a cursor while pressing the left mouse button) at the level of 10-20 % of maximum fluorescence obtained for the Positive Controls in the last amplification cycle. Make sure that fluorescence curve of the Positive Control crosses the threshold line at the zone of exponential growth of fluorescence passing onto linear growth.

Variant 2

For each channel indicate **Log Scale** with a checkmark. Set the threshold line at the level where fluorescence curves are linear (use the left mouse button).

3. Click the **View/Edit Plate** button on the toolbar and enter sample names in the opened window, if required.

Click **Tools** on the toolbar, then **Reports…**, and then save the generated report choosing **File** and **Save As** and name the file.

NOTE: Results interpretation for controls and for clinical material must be carried out in compliance with the *Instruction Manual* and *Important Product Information Bulletin*.

List of Changes Made in the Guidelines

VER	Location of changes	Essence of changes
25.02.15 PM	Text	Text was corrected in accordance with the template
	Intended use	The CFX96 (Bio-Rad, USA) instrument 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 using SmartCycler (Cepheid, USA)	The amplification programs was added
	Amplification and data analysis using CFX96 (Bio-Rad, USA)	The chapter was added
29.12.20 VA	Through the text	The symbol ⚠ was changed to NOTE:
	Cover page	The phrase "Not for use in the Russian Federation" was added
16.03.21 MA	Cover page	The name, address and contact information for Authorized representative in the European Community was changed