

GUIDELINES

to AmpliSens[®] *N.meningitidis* / *H.influenzae* / *S.pneumoniae*-FRT PCR kit

for qualitative detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* DNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection

AmpliSens[®]



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

TABLE OF CONTENTS

| | |
|---|----|
| INTENDED USE | 3 |
| AMPLIFICATION AND DATA ANALYSIS USING Rotor-Gene 3000/6000 (Corbett Research, Australia) AND Rotor-Gene Q (QIAGEN, Germany) INSTRUMENTS | 3 |
| AMPLIFICATION AND DATA ANALYSIS USING iCycler iQ5 (Bio-Rad, USA) INSTRUMENT | 7 |
| AMPLIFICATION AND DATA ANALYSIS USING CFX96 (Bio-Rad, USA) | 10 |
| TROUBLESHOOTING | 13 |

INTENDED USE

The guidelines describe the procedure of using **AmpliSens® *N.meningitidis* / *H.influenzae* / *S.pneumoniae*-FRT** PCR kit for qualitative detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* DNA in the biological material (cerebrospinal fluid) 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);
- CFX96 (Bio-Rad, USA);
- iCycler iQ5 (Bio-Rad, USA).

Correspondence of the fluorophores and detection channels

| Channel for the fluorophore | Detection channel name for different instrument models ¹ |
|-----------------------------|---|
| FAM | FAM/Green |
| JOE | JOE/HEX/R6G/Yellow/Cy3 |

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 instrument.

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

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 flat caps (e.g. Axygen, USA), or 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).

Programming the thermocycler

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

¹ The detection channels names in each section of the guidelines are specified in accordance with the described instrument.

Well 1 must be filled with any test tube except for an empty one. If the tubes with reagents from different PCR kits or with different PCR-mixes are inserted into the rotor then the tubes' numbers for calibration in each detection channel should be indicated in the Rotor-Gene software. Recommendations about the calibration are described in the information list "Calibration priorities for the AmpliSens kits for Rotor-Gene 3000/6000 (Corbett Research, Australia) and Rotor-Gene Q (QIAGEN, Germany) thermocyclers".

Creating the template for the run

1. Click the **New** button in the software main menu. To create the template select the **Advanced** tab in the opened window **New run**.
2. Select the **TwoStep/Hidrolysis Probes** template in the tab for edition and click The **New** button.
3. In the opened window select the **36-Well Rotor (or 72-Well Rotor)** and tick the **No Domed 0,2ml Tubes / Locking Ring Attached** option. Click the **Next** button.
4. In the opened window enter the operator name, select the **Reaction volume – 25 µl**. Tick the **15 µl oil layer volume** option. Click the **Next** button.
5. In the **New Run Wizard** window set the temperature profile of the experiment. To do this click the **Edit profile** button and set the amplification program:

Amplification program

| Step | Temperature, °C | Time | Fluorescence detection | Number of cycles |
|-------------|------------------------|-------------|-------------------------------|-------------------------|
| 1 | 95 | 15 min | – | 1 |
| 2 | 95 | 10 s | – | 45 |
| | 56 | 20 s | FAM/Green, JOE/Yellow | |
| | 72 | 10 s | – | |

Note – The ROX/Orange, Cy5/Red, Cy5.5/Crimson channels are enabled when required if the "multiprime" format tests are performed.

6. After setting up the temperature profile click the **OK** button.
7. Set calibration parameters (click the **Calibrate/Gain Optimisation...** button in a new experiment module):
 - Perform fluorescence measurement in FAM/Green and JOE/Yellow channels (activate **Calibrate Acquiring/Optimise Acquiring**);
 - Perform calibration before the first measurement in FAM/Green and JOE/Yellow channels (activate **Perform Calibration Before 1st Acquisition/Perform Optimisation Before 1st Acquisition**);
 - Set channel calibrations: for FAM/Green channel by the first tube and from 5FI to 10FI; for JOE/Yellow channel, from 5 FI to 10FI (activate the **Edit...** button, **Auto gain calibration channel settings** window);

8. Select the **Start run** button for amplification run and name the experiment.
9. During or after amplification, it is necessary to program the order of the test samples, Negative Control of Extraction, Positive and Negative Controls of Amplification. Enter the data in 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 Positive Control of Amplification as **C+**, Negative Control of Amplification as **C-**. Set the **Unknown** type opposite all test samples, the **Positive Control** type – for the Positive Controls, the **Negative Control** type - for the Negative Control of Extraction, and the **NTC** type next to the Negative Control of Amplification. Set the type **None** for the cells matching with the corresponding empty tubes.

Data analysis

The obtained results are analyzed by the Rotor-Gene software. The results are interpreted according to the crossing (or not-crossing) of the S-shaped (sigmoid) fluorescence curve with the threshold line set at the specific level, that corresponds to the presence (or absence) of the *Ct* (threshold cycle) value of the DNA sample in the corresponding column of the results table.

Amplification data analysis in the FAM/Green and JOE/Yellow channel channels:

1. Activate the button **Analysis** in the menu, select the mode of the analysis **Quantitation**, activate the **Cycling A. JOE/Cycling A. Yellow, Show** and **Cycling A. FAM/Cycling A. Green, Show** buttons.
2. Cancel the automatic choice of the threshold line level **Threshold** for each opened main window (FAM/Green and JOE/Yellow).
3. Select the **Linear scale**.
4. Activate the **Dynamic tube** and **Slope Correct** buttons in the menu of main window (**Quantitation analysis**).
5. In the **CT Calculation** menu (in the right part of window) indicate the threshold line **Threshold = 0.05**.
6. Choose the parameter **More settings/Outlier Removal** and set the value of negative samples threshold (**NTC/Threshold**) as **10 % for FAM/Green channel or 5-10% for JOE/Yellow channel**.
7. In the results grid (the **Quant. Results** window) one will be able to see the *Ct* values.

Results interpretation

The result of the PCR analysis is considered reliable only if the results for the controls of the amplification and the extraction are correct in accordance with the table of assessment

of results for controls (see the *Instruction manual*) and boundary values specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The interpretation of the test samples is to be carried out in accordance with the *Instruction Manual* and the *Important Product Information Bulletin* enclosed to the PCR kit.

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 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).

Programming the thermocycler


1. Turn on the instrument and the power supply unit of the optical block of the instrument.

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

2. Start the program iCycler iQ5.
3. Insert the tubes or strips into the reaction module of the thermocycler and program the instrument according to the *Instruction Manual* given by the manufacturer of 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 tubes (strips) upside down while inserting them into the instrument.

Creating the template for the run

1. Set the plate setup (set the order of the tubes in the reaction chamber and the detection of fluorescent signal).
 - click the **Create New** button in the **Selected Plate Setup** window of the **Workshop** module;
 - in the opened window click the **Whole Plate loading** button and set the plate setup using the buttons of the upper toolbar. Enter the samples' names in the **Identifier/Condition** column in the bar appeared in the screen bottom. Select the fluorescent signal detection in the FAM, JOE/HEX channels. Click the **Select/Add Fluorophores** button, select the fluorophore and tick it in the **Selected** column. Click **OK**. The fluorophore name will appear in the **Fluorophore** window. For addition of fluorescence signal measuring for each sample it is necessary to click the fluorophore (activate it) and select the samples on the plate using the **Fluorophore loading in whole Plate mode**  button under the scheme;
 - set the reaction volume (**Sample Volume**) as **25 µl**, 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. Enter the

file name and click **Save**.

2. Set all the biological samples as **Unknown**, positive controls as «+», and negative controls as «-».
3. Set the amplification program. To do this, in the **Selected Protocol** window of the **Workshop** module click the **Create New** button. Set the amplification parameters and save the protocol by activating the **Save&Exit Protocol Editing** button. Enter the name of the file and then click **Save**.

Amplification program

| Step | Temperature, °C | Time | Fluorescence detection | Number of cycles |
|------|-----------------|--------|------------------------|------------------|
| 1 | 95 | 15 min | – | 1 |
| 2 | 95 | 10 s | – | 45 |
| | 56 | 25 s | FAM, JOE/HEX | |
| | 72 | 10 s | – | |

Note – The ROX, Cy5, Cy5.5 channels are enabled when required if the “multiprime” format tests are performed.

4. Before a run it is obligatory to check if the selected protocol (**Selected Protocol**) and the plate scheme (**Selected Plate Setup**) are correct. To begin a run click the **Run** button. For the well factors measurement the **Use Persistent Well Factors** type is selected by default. Click the **Begin Run** button, save the experiment (the results of this experiment will be automatically saved in this file) and click **OK**.
5. At the end of the run it is necessary to close the software and turn off the instrument (the thermocycler and the optical block).

Using the ready template for the run

The test parameters and the plate setup set earlier can be used for the further runs. To do this:

- select the needed file with the run in the upper left window of the **Workshop** module;
- click the **Edit** button in the **Selected Plate Setup** area of the **Workshop** module and edit the plate setup (the files of protocols are saved in the **SampleFiles** folder by default);
- click the **Edit** button in the **Selected Protocol** area of the **Workshop** module and check the correctness of the selected protocol (the files of protocols are saved in the **Users**) folder by default).

Data analysis:

The obtained results are analyzed by the iCycler iQ5 software. The results are interpreted according to the crossing (or not-crossing) of the S-shaped (sigmoid) fluorescence curve with the threshold line set at the specific level, that corresponds to the presence (or

absence) of the *Ct* (threshold cycle) value in the corresponding column of the results table.

1. Start the software and open the needed file with data of the analysis in the **Data File** window of the **Workshop** module. Click the **Analyze** button.
2. Select the **Analysis Mode: PCR Base Line Subtracted Curve Fit** (is set by default).
3. Check the correctness of threshold line automatic choice for each channel. The threshold line is to cross only with S-shaped (sigmoid) curves describing the accumulation 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 set the threshold line level for each channel manually. To do this, click the **Log View** (logarithmic scale selection) and set (with the left mouse button) the threshold line at the level where the fluorescence curves have a linear character and do not cross with the curves of the negative samples. As a rule, the threshold line is set at the level of 10-20 % of maximum fluorescence obtained for the Positive control in the last amplification cycle. Make sure that the fluorescence curve of the Positive control has the typical exponential growth of fluorescence.
4. In order to analyze the results click the **Results** button which is situated under the buttons with the fluorophores' names

Results interpretation

The result of the PCR analysis is considered reliable only if the results for the controls of the amplification and the extraction are correct in accordance with the table of assessment of results for controls (see the *Instruction manual*) and boundary values specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The interpretation of the test samples is to be carried out in accordance with the *Instruction Manual* and the *Important Product Information Bulletin* enclosed to the PCR kit.

AMPLIFICATION AND DATA ANALYSIS USING CFX96 (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 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).

Programming the thermocycler

1. Turn on the instrument and start the Bio-Rad CFX Manager software.
2. Program the instrument according to the *Instruction Manual* provided by the manufacturer.

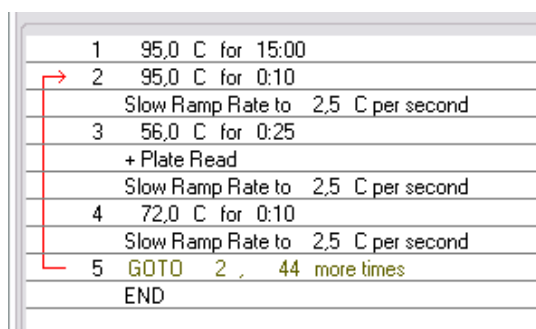
Creating the template for the run

1. In the **Startup Wizard** window it is necessary to select the **Create a new Run/Experiment** (or select **New** in the **File** menu and then select **Run.../Experiment...**). Click **OK**.
2. In the **Run Setup** window, select **Protocol** tab and click the **Create new...** Set the amplification parameters (time, temperature, cycles, and fluorescence acquiring cycle) in the opened **Protocol Editor – New** window. Set **Sample Volume – 25 µl**.

Amplification program

| Step | Temperature, °C | Time | Fluorescence detection | Number of cycles |
|------|-----------------|--------|------------------------|------------------|
| 1 | 95 | 15 min | – | 1 |
| 2 | 95 | 10 s | – | 45 |
| | 56 | 25 s | FAM, HEX | |
| | 72 | 10 s | – | |

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



| | |
|---|------------------------------------|
| 1 | 95,0 C for 15:00 |
| 2 | 95,0 C for 0:10 |
| | Slow Ramp Rate to 2,5 C per second |
| 3 | 56,0 C for 0:25 |
| | + Plate Read |
| | Slow Ramp Rate to 2,5 C per second |
| 4 | 72,0 C for 0:10 |
| | Slow Ramp Rate to 2,5 C per second |
| 5 | GOTO 2, 44 more times |
| | END |

Note – The ROX, Cy5, Cy5.5 channels are enabled when required if the “multiprime” format tests are performed.

3. Save the protocol: in the **Protocol Editor New** window select **File**, then **Save As**, name the file and click **Save**.

4. Set the plate setup: in the **Plate** tab click the **Create new...** button. Set the tube order in the opened **Plate Editor – New** window. Click the **Select Fluorophores...** button and click the **Selected** checkbox next to the **FAM, HEX** fluorophores and click **OK**. In the **Sample type** menu select **Unknown** for all the samples. Then in the **Load** column (in the right part of the window) tick the fluorescence signal acquiring for all the samples in the required channels. Define sample names in the **Sample name** window, moreover the **Load** function is to be ticked.
5. Save the plate setup: select **File** and then **Save as** in the **Plate Editor New** window. Enter the file name, click **Save**.
6. Select the **Start Run** tab. Open the lid of the instrument by the **Open Lid** button. Insert the reaction tubes in the wells of the instrument in accordance with the entered plate setup. Close the lid by the **Close Lid** button.

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 tubes (strips) upside down while inserting them into the instrument.

7. Click the **Start Run** button and start the program with the selected plate setup. Select the directory for the file saving, name the file, click **Save**.

Using the ready template for the run

The test parameters and the plate setup set earlier can be used for the further runs. To do this:

- click the **Select Existing...** button in the **Run Setup** window of the **Protocol** tab. Select the needed file with the amplification program in the **Select Protocol** window. Click **Open**.
- go to the **Plate** tab in the **Run Setup** window. Click the **Select Existing...** button. Select the needed file with the plate setup in the **Select Plate** window. Click **Open**. Click the **Edit selected** button to edit the plate setup.

Data analysis

The obtained results are analyzed by the software of the CFX96 instrument. The results are interpreted according to the crossing (or not-crossing) of the S-shaped (sigmoid) fluorescence curve with the threshold line set at the specific level, that corresponds to the presence (or absence) of the *Ct* (threshold cycle) value in the corresponding column of the results table.

1. Start the software and open the saved file with data of the analysis. To do this, select **File** in the menu, then **Open** and **Data file** and select the needed file.
2. The fluorescence curves, the tube order in the plate and the table with the *Ct* values are represented in the **Data Analysis** window of the **Quantification** tab.

Check the correctness of threshold line automatic choice for each channel. The threshold line is to cross only with S-shaped (sigmoid) curves describing the accumulation 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 set the threshold line level for each channel manually. To do this, tick the **Log Scale** item (logarithmic scale selection) and set (with the left mouse button) the threshold line at the level where the fluorescence curves have a linear character and do not cross with the curves of the negative samples. As a rule, the threshold line is set at the level of 10-20 % of maximum fluorescence obtained for the Positive control in the last amplification cycle. Make sure that the fluorescence curve of the Positive control has the typical exponential growth of fluorescence. To select the curve of C+ sample (or another one appropriate sample), set the cursor to the plate setup or to the results grid.

Results interpretation

The result of the PCR analysis is considered reliable only if the results for the controls of the amplification and the extraction are correct in accordance with the table of assessment of results for controls (see the *Instruction manual*) and boundary values specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The interpretation of the test samples is to be carried out in accordance with the *Instruction Manual* and the *Important Product Information Bulletin* enclosed to the PCR kit.


TROUBLESHOOTING

1. If the *Ct* value determined for the Positive Controls of Amplification (C+) in the channels for the FAM or JOE fluorophores is greater than the boundary *Ct* value, the amplification and detection should be repeated for all samples in which *Ct* value determined in the channels for the FAM or JOE fluorophores was greater than the boundary *Ct* value for required PCR-mix-1.
2. If the *Ct* value determined for the Negative Control of Extraction (C-) (except for PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI in the channel for FAM fluorophore) and/or Negative Control of Amplification (NCA) (in all channels) is less than the boundary *Ct* value, PCR-analysis should be repeated (beginning with DNA extraction) for all samples in which target pathogen DNA was detected.
3. If the *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.
4. In case of simultaneous amplification using two PCR-mixes (PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI and PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*) in one instrument and if the following results are obtained for the test sample, it is necessary to do:

| No | PCR-mix-1 | FAM/Green | JOE/Yellow/ HEX | Recommended action |
|----|---|-----------------------------|-----------------------------|---|
| 1 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | > boundary value | > boundary value | The analysis (beginning with the DNA extraction stage) must be repeated for the sample |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | < boundary value | > boundary value | |
| 2 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | > boundary value | > boundary value | The analysis (beginning with the DNA extraction stage) must be repeated for the sample |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | > boundary value | < boundary value | |
| 3 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | > boundary value | < boundary value | The analysis (beginning with the DNA extraction stage) must be repeated for the sample due to the atypical combination of pathogens |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | < boundary value | > boundary value | |
| 4 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | > boundary value | < boundary value | The analysis (beginning with the DNA extraction stage) must be repeated for the sample due to the atypical combination of pathogens |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | > boundary value | < boundary value | |

| № | PCR-mix-1 | FAM/Green | JOE/Yellow/ HEX | Recommended action |
|----------|---|-----------------------------|-----------------------------|---|
| 5 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | > boundary value | > boundary value | The analysis (beginning with the DNA extraction stage) must be repeated for the sample due to the atypical combination of pathogens |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | < boundary value | < boundary value | |
| 6 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | < boundary value | < boundary value | The analysis (beginning with the PCR stage) must be repeated for the sample due to the atypical combination of pathogens |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | < boundary value | > boundary value | |
| 7 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | < boundary value | < boundary value | The analysis (beginning with the PCR stage) must be repeated for the sample due to the atypical combination of pathogens |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | > boundary value | < boundary value | |
| 8 | PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI | < boundary value | > boundary value | The analysis (beginning with the PCR stage) must be repeated for the sample due to the atypical combination of pathogens |
| | PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i> | < boundary value | < boundary value | |

List of Changes Made in the Guidelines

| VER | Location of changes | Essence of changes |
|--|---|--|
| 27.06.11 RT | Cover page | The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology” |
| 02.04.14 SA | Cover page | Address of European representative was added |
| 20.08.15 PM | Through the text | Corrections according to the template |
| 17.02.16 PM | Through the text | Corrections according to the template |
| | Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia) AND Rotor-Gene Q (QIAGEN, Germany) instruments | The Rotor-Gene Q instrument was added |
| | | The amplification program was added |
| | The value for the parameter More settings/Outlier Removal was changed from 10 % to the 5-10 % range for JOE/Yellow channel | |
| Amplification and data analysis using iCycler iQ5 (Bio-Rad, USA) instruments | The amplification program was added | |
| 10.04.18 PM | Amplification and data analysis using CFX96 (Bio-Rad, USA) | The sections has been added |
| | Troubleshooting | |
| 10.04.18 PM | Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia) and Rotor-Gene Q (QIAGEN, Germany) instruments | The amplification program was specified |
| 07.06.21 EM | Through the text | The symbol  was changed to NOTE: |
| | Cover page | The phrase “For research use only. Not for diagnostic procedures” was added |
| 14.07.23 EM | Footer | REF R-B25(RG,iQ)-CE was added |