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For Professional Use Only

GUIDELINES

to **AmpliSens®** ***Rubella virus*-FRT** PCR kit

for qualitative detection of *Rubella virus* RNA in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection

AmpliSens®

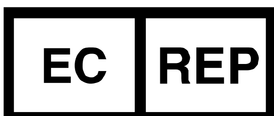

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TABLE OF CONTENTS

INTENDED USE 3
WORK with the NucliSENS easyMAG AUTOMATED NUCLEIC ACID EXTRACTION SYSTEM 3
AMPLIFICATION AND DATA ANALYSIS USING Rotor-Gene 3000/6000 (Corbett Research, Australia) INSTRUMENT 5
AMPLIFICATION AND DATA ANALYSIS USING iCycler iQ5 and iCycler iQ (Bio-Rad, USA) INSTRUMENTS 8
AMPLIFICATION AND DATA ANALYSIS USING Mx3000P, Mx3005P (Stratagene, USA) INSTRUMENT 11

INTENDED USE

The guidelines describe the procedure of using **AmpliSens® *Rubella virus*-FRT** PCR kit for qualitative detection of *Rubella virus* RNA in the clinical material (peripheral and umbilical cord blood plasma, saliva, oropharyngeal swabs, and amniotic 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);
- iCycler iQ, iCycler iQ5 (Bio-Rad, USA);
- Mx3000P, Mx3005P (Stratagene, USA).

and also in combination with the automatic station for the nucleic acids extraction NucliSENS easyMAG (bioMérieux, France).

WORK with the NucliSENS easyMAG AUTOMATED NUCLEIC ACID EXTRACTION SYSTEM

Variant 1

RNA extraction with lysis of sample outside of the instrument (off-board mode)

This method of extraction allows reducing the consumption of NucliSens lysis buffer. It is preferred for working with clinical samples which contain clots (saliva).

1. Switch on the NucliSENS easyMAG instrument and prepare it to the RNA extraction according to the instruction manual.
2. In the window for input of test samples, enter the following parameters:
   - Sample name
   - ***Matrix*** for RNA extraction (select ***Other***)
   - ***Volume*** – ***0.1 ml***
   - ***Eluate*** – ***55 µl***
   - ***Type*** – ***Lysed***
   - ***Priority*** – ***Normal***.
3. Create a new protocol of RNA extraction and save it. In the protocol select ***On-board Lysis Buffer Dispensing*** - ***No***, ***On-board Lysis Incubation*** - ***No***.
4. Relocate the sample table into the created protocol.
5. Take the required quantity of special disposable tubes intended for RNA extraction in the NucliSENS easyMAG instrument (including Negative Control of extraction). Add **10 µl** of **Internal Control STI-87** to inner walls of each tube and then add **550 µl** of **NucliSens lysis buffer**.

When working with material which contains clots, lysis should be carried out in 1.5-ml tubes. After finishing the incubation (see item 8), tubes should be centrifuged at 10,000 rpm for 1 min. Then transfer the supernatant into special tubes intended for RNA/DNA extraction in the NucliSENS easyMAG instrument

6. Add **100 µl** of prepared samples into each tube with **Lysis buffer** and **Internal Control STI-87-rec** by using disposable tips with aerosol barriers and carefully mix by pipetting (avoid getting mucus clots and big particles into the tube).
7. Add **100 µl** of **Negative Control** into the tube with the Negative Control of Extraction (C–). Add **90 µl** of **Negative Control** and **10 µl** of **Positive Control *Rubella virus*-rec (PCE)** into the tube with the Positive Control of Extraction (PCE).
8. Incubate the tubes for 10 min at room temperature.
9. Resuspend the tube with **magnetic silica NucliSens** (bioMérieux) by intensive vortexing. Add **25 µl** of **magnetic silica** by using disposable tips with aerosol barriers and carefully mix by pipetting. Magnetic silica should be distributed evenly over the tube volume.
10. Place the tubes with samples into the instrument and start the RNA extraction program with lysis of samples by selecting the ***Off-board*** mode.
11. After the extraction is finished, take the tubes out of the instrument and carry out the RT-PCR not later than 30 min after RNA extraction.

If necessary to store, transfer purified RNA into sterile tubes within 30 min after RNA extraction.

The purified RNA should be stored at 2–8° C for 8 hours, at the temperature ≤ –16 °C for 1 month, and at the temperature ≤ –70° C for a long time.

Variant 2

RNA extraction with automated lysis of sample in the instrument (on-board mode)

1. Switch on the NucliSENS easyMAG instrument and prepare it for the RNA extraction according to the instruction manual.
2. In the window for input of test samples, enter the following parameters:
   - Sample name
   - ***Matrix*** for RNA extraction (select ***Other***)
   - ***Volume*** –0.1 or 1 ml
   - ***Eluate*** – 55 µl
   - ***Type*** – ***Primary***
   - ***Priority*** – ***Normal***.
3. Create a new protocol of RNA extraction and save it. In the protocol, select ***On-board***

Lysis Buffer Dispensing - ***Yes***, ***On-board Lysis Incubation*** - ***Yes***.

4. Relocate the programmed sample into the created protocol.
5. Take the required quantity of special disposable tubes intended for RNA extraction in NucliSENS easyMAG instrument (including negative control of extraction). Add **10 µl** of **Internal Control STI-87-rec** into each tube along the internal walls.
6. Add **100 µl** of prepared samples into each tube with **lysis buffer** and **Internal Control** by using disposable tips with aerosol barriers and carefully mix by pipetting (avoid getting mucus clots and big particles into the tube).
7. Add **100 µl** of **Negative Control** into the tube with Negative Control of Extraction (C-). Add **90 µl** of **Negative Control** and **10 µl** of **Positive Control *Rubella virus*-rec (PCE)** into the tube with the Positive Control of Extraction (PCE).
8. Place the tubes with samples into the instrument, place the tips and run the RNA extraction with lysis of samples in the instrument (the ***On-board*** mode).
9. Wait until the NucliSENS easyMAG instrument proceeds to the ***Instrument State–Idle*** option and pauses.
10. Thoroughly vortex the tube with **magnetic silica NucliSens** (bioMérieux). Open the lid of instrument and add **25 µl** of **magnetic silica** into each tube by using disposable tips with aerosol barriers (or by using multichannel pipettes with disposable tips with aerosol barriers for **200 µl**) and carefully mix by pipetting. Magnetic silica should be distributed evenly over the tube volume.
11. Close the lid of the instrument and continue the RNA isolation program.
12. After the extraction is finished, take the tubes out of the instrument and carry out the RT-PCR not later than 30 min after RNA extraction.

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

Insert the tubes into the rotor of the Rotor-Gene 3000/6000 instrument beginning from the first well (the rotor wells are numbered, the numbers are used for the further programming of the samples' order in the thermocycler). Insert the rotor into the instrument, close the lid.

If some wells of the rotor remain unloaded, the rotor should be balanced. Place empty tubes into the empty wells. Do not use tubes from the previous tests. Well 1 must be filled with any test tube except for an empty one.

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

Programming the Rotor-Gene 3000/6000 instrument

1. Click the ***New*** button in the software main menu.
2. In the window, select the ***Advanced*** menu and select ***Dual Labeled Probe/Hydrolysis probes***. Click the ***New*** button.
3. In the opened window, select ***36-Well Rotor*** (or ***72-Well Rotor***) and ***No Domed 0.2 ml Tubes/Locking ring attached***. Click the ***Next*** button***.***
4. In the opened window enter the operator name, and select the reaction mixture volume: ***Reaction volume*** – **25 µl.** Select ***15 µl oil layer volume***. Press the ***New*** button.
5. In the opened window set the temperature profile of the experiment. To do this click the ***Edit profile*** button and set the amplification program::

Table 1

AmpliSens-2 amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Number of cycles***
Hold	50	15 min	–	1
Hold 2	95	15 min	–	1
Cycling	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 s	–	

It is possible to carry out any combination of tests that use the AmpliSens-2 universal amplification program (for example, *HCV*-genotyping, etc.) within the same run. ROX/Orange and Cy5/Red channels are activated if necessary (for "multiprime" format tests).

6. After setting up the temperature profile click the ***OK*** button.
7. In the ***New Run Wizard*** window, click the ***Calibrate*** or ***Gain Optimisation*** button.

– Perform calibration in **FAM/Green** and **JOE/Yellow** channels (press the ***Calibrate Acquiring/Optimise Acquiring*** button).
– Perform calibration before the first measurement (***Perform Calibration Before 1st Acquisition*** or ***Perform Optimisation Before 1st Acquisition)***.
– Set the calibration of the channels for all dyes from 3Fl to 8Fl (***Edit…*** button, ***Auto gain calibration channel settings*** window). Press the ***Close*** button.

8. Click ***Next***. Then press the ***Start run*** button for amplification run.
9. Name the experiment and save it to the disk (results of the experiment will be automatically saved in this file).
10. Enter the data in the grid of the samples (it opens automatically after amplification has been started). Enter the names/numbers of the test samples in the ***Name*** column. Define the Negative Control of amplification as ***NCA*** and the Positive Control of Amplification as ***C+***. Set the type ***Unknown*** opposite all the tested samples, the type ***Positive Control*** - for the Positive Control of amplification, the type ***Negative Control*** - for the Negative Control of Amplification. Set the type ***None*** for the cells matching with the corresponding empty tubes.

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

Data analysis:

The obtained results (fluorescence accumulation curves for two channels) are analyzed by the instrument software. Accumulation of the **Internal Control STI-87-rec cDNA** amplification product is detected in the **FAM/Green** channel, ***Rubella virus*** **cDNA** is detected in the **JOE/Yellow** channel.

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 cDNA sample in the corresponding column of the results table.

Amplification data analysis for the *Rubella virus* cDNA (JOE/Yellow channel).

1. Activate the button ***Analysis*** in the menu, select the mode of the analysis ***Quantitation***, activate the ***Cycling A. FAM/Cycling A. Green***, ***Show*** buttons.
2. Cancel the automatic choice of the threshold line level ***Threshold***.
3. Activate the ***Dynamic tube*** and ***Slope Correct*** buttons in the menu of main window (***Quantitation analysis***).
4. In the ***CT Calculation*** menu (in the right part of the window), indicate the threshold line

level **0.03** in the ***Threshold*** box.

5. Choose the parameter ***More settings/Outlier Removal*** and set **10 %** for the value of negative samples threshold (***NTC/Threshold***).
6. In the results grid (the ***Quantitation Results*** window) one will be able to see the *Ct* values.
7. For Negative Control of Extraction (C-) – **Negative Control (C-)** - *Ct* values should be absent.
8. For Negative Control of Amplification (NCA) – **RNA-buffer** – *Ct* values should be absent.
9. For the Positive Control of Extraction – **Positive Control cDNA *Rubella virus* / STI (C+)** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin*.
10. For Positive Control of Amplification – **Positive Control *Rubella virus*-rec** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin*.

Amplification data analysis for IC (FAM/Green channel).

1. Activate the button ***Analysis*** in the menu, select the mode of the analysis ***Quantitation***, activate ***Cycling A. FAM/Cycling A. Green***, ***Show*** buttons.
2. Cancel the automatic choice of the threshold line level ***Threshold***.
3. Activate the ***Dynamic tube*** and ***Slope Correct*** buttons in the menu of main window (***Quantitation analysis***).
4. In the ***CT Calculation*** menu (in the right part of the window), indicate the threshold line level **0.03** in the ***Threshold*** box.
5. Choose the parameter ***More settings/Outlier Removal*** and set **10 %** for the value of negative samples threshold (***NTC/Threshold***).
6. In the results grid (the ***Quant. Results*** window), one will be able to see the *Ct* values for **Internal Control STI-87 (IC)** for each test sample.
7. For Negative Control of Amplification (NCA) – **RNA-buffer** – *Ct* values should be absent.
8. For the Positive Control of Extraction – **Positive Control cDNA *Rubella virus* / STI (C+)** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin*.
9. For Negative Control of Extraction (C-) – **Negative Control (C-)** – *Ct* values should not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin*.

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

1. Turn on the instrument and start the iQ5 program.

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

2. Insert the tubes, strips (part of the plate) or the plate into the reaction module. Program the instrument.

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.

Program the thermocycler according to manufacturer's manual.

1. Click the ***Create New*** button in the ***Workshop*** module to create a new protocol.
2. Set the amplification parameters in the opened window (see Table 2).

Table 2

AmpliSens-2 amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Number of cycles***
1	50	15 min	–	1
2	95	15 min	–	1
3	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
4	95	5 s	–	40
	60	20 s	FAM, JOE/HEX, ROX, Cy5	
	72	15 s	–	

It is possible to carry out any combination of tests that use the AmpliSens-2 universal amplification program (for example, *HCV*-genotyping, etc.) within the same run. ROX and Cy5 channels are activated if necessary (for "multiprime" format tests).

3. Name the new protocol and save it.
4. Create the new plate of samples - ***Plate setup***. Set the order of the tubes in the plate.
5. In the open window, mark all clinical samples as ***Unknown,*** positive controls as «**+**», and negative controls as «**–**». Set the fluorescence measurement in 2 channels (**HEX-530** and **FAM-490)** for all samples.
6. Name the scheme of the tubes order and save it
7. Click the ***Run*** button (for iCycler iQ5) or the ***Run with selected protocol*** button (for iCycler iQ). Set the volume of the sample as **25 µl.** For iCycler iQ, select ***Experimental Plate*** to determine the well factor. For iCycler iQ5, both the mode with measurement of well factors by experimental tubes and the mode with fixed well factors (recommended)

can be used. Click the ***Begin Run*** button and save the experiment.

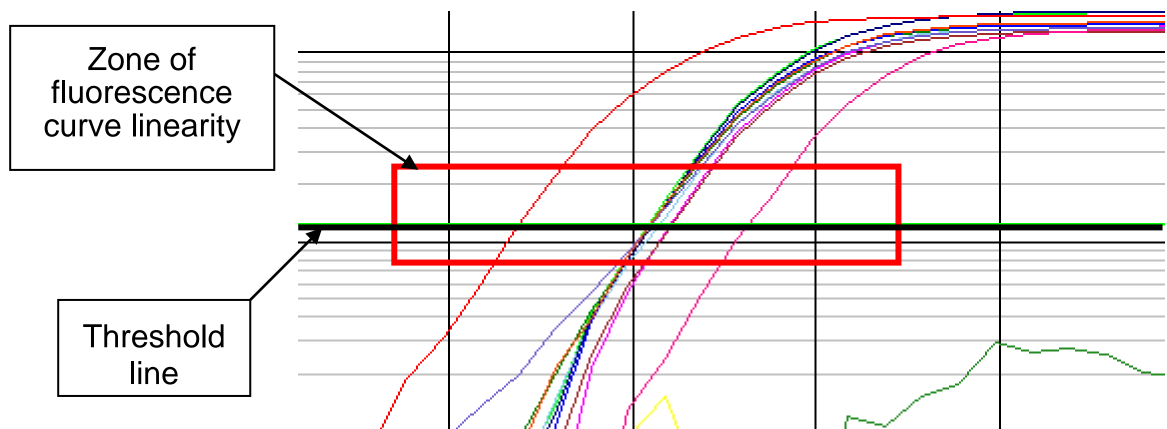
Data analysis

The obtained results (fluorescence accumulation curves for two channels) are analyzed by the instrument software. Accumulation of the **Internal Control STI-87-rec cDNA** amplification product is detected in FAM channel, ***Rubella virus* cDNA** – in the **JOE/HEX channel**.

The results are interpreted according to the crossing (or not-crossing) of the 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.

Data processing

1. Start the software and open the saved file: select ***Data file*** in **the *Workshop*** module and select the data file. Proceed to the ***Data Analysis*** mode.
2. View data separately for each channel.
3. Select ***Log View*** for each channel. Set the threshold line (with the left mouse button) at a level where fluorescence curves have a linear character.



4. In order to analyze the results click the ***Results*** button which is situated under the buttons with the fluorophores' names (for **iCycler iQ5**) or ***PCR Quant*** button (for **iCycler iQ**). In the results grid (***Quant. Results*** window) the *Ct* values for each channel will appear.

Amplification data analysis for the *Rubella virus* cDNA (JOE/HEX channel).

1. Microsoft Excel window will open. In the results grid the *Ct* values will appear (column Ct (dR)).
2. For Negative Control of Extraction (C-) – **Negative Control (C-)** – *Ct* values should be absent.

3. For Negative Control of Amplification (NCA) – **RNA-buffer** – *Ct* values should be absent.
4. For the Positive Control of Extraction – **Positive Control cDNA *Rubella virus* / STI (C+)** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin*.
5. For Positive Control of Amplification – **Positive Control *Rubella virus*-rec** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin.*

Amplification data analysis for the IC (FAM channel).

1. Press the ***FAM*** button in ***Dyes Shown*** window.
2. Select ***Text Report*** in ***Area to analyze.*** Open the ***File*** menu, then select ***Export Text Report*** and ***Export Text Report to Excel***. **Ct** values will appear in the column Ct (dR).
3. For Negative Control of Amplification (NCA) – **RNA-buffer** – *Ct* values should be absent.
4. For the Positive Control of Extraction – **Positive Control cDNA *Rubella virus* / STI (C+)** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin*.
5. For Negative Control of Extraction (C-) – **Negative Control (C-)** – *Ct* values should be less than the value specified in the *Important Product Information Bulletin*.

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

1. Switch on the instrument. Run the software Stratagene Mx3000P.
2. Select ***Quantitative PCR (Multiple Standards)*** and ***Turn lamp on for warm-up*** in ***New Experiment Options*** window.

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

3. Insert the tubes into the instrument and close the lid.
4. Select ***Optics Configuration*** in the ***Options*** menu and in ***Dye Assignment*** tab set **JOE** parameters opposite to the ***HEX/JOE filter set*** item, **FAM** parameters in front of the ***FAM filter set*** item.

Don’t turn the strips/plate upside down while inserting them into the instrument.

5. Lock the fixing arm and the door of the instrument
6. Select ***Quantitative PCR (Multiple Standards)*** in ***New Experiment Options*** window and set ***Turn lamp on for warm-up***.
7. Set fluorescence detection parameters in the ***Plate Setup*** menu. For this:
   – Select all wells with the test tubes or strips (hold ***Ctrl*** button down and select the necessary region with the mouse).
   – Mark all selected cells as ***Unknown*** in the ***Well type*** window. Set **FAM** and **JOE** in ***Collect fluorescence data*** option. Name each sample by double clicking on each cell (***Well Information*** window).
8. Set fluorescence detection parameters for tubes in the ***Plate Setup*** tab. For this:
   – Select all cells with analysis tubes or strips (holding ***Ctrl*** down and selecting the required range)
   – Mark all selected cells as ***Unknown*** in the ***Well type*** window. Set **FAM** and **JOE** in ***Collect fluorescence data*** option. Name each sample by double clicking on each cell (***Well Information*** window), positive control indicate as **+**, negative - as **-**.
9. In the ***Thermal Profile Setup*** tab, set the amplification program according to the one of this modes:

<u>Using of the template file</u> for setting the amplification program (is recommended).

Press the ***Import…*** button right to thermocycling profile picture. Proceed to the folder containing previous experimental file and open it. In the ***Thermal Profile*** window required thermocycling profile will appear.

Individual programming.

1. After setting all necessary values and parameters, select all wells with tested microtubes again. Proceed to ***Thermal Profile Setup*** menu, set the amplification program specified in the Table 3.

Table 3

AmpliSens-2 amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Number of cycles***
1	50	15 min	–	1
2	95	15 min	–	1
3	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
4	95	5 s	–	40
	60	20 s	FAM, JOE/HEX, ROX, Cy5	
	72	15 s	–	

It is possible to carry out any combination of tests that use the AmpliSens-2 universal amplification program (for example, *HCV*-genotyping, etc.) within the same run. ROX and Cy5 channels are activated if necessary (for “multiprime” format tests).

2. To set detection parameter of fluorescent signal at desired temperature, select the ***All points*** option for ***Data collection by marker dragging*** parameter and move it by mouse from right side to the shelf with desired temperature.
3. Select ***Run*** and ***Start*** and name the experiment file.

Data analysis

The obtained results (fluorescence accumulation curves for two channels) are analyzed by the instrument software. Accumulation of the **Internal Control STI-87-rec cDNA** amplification product is detected in **FAM** channel, ***Rubella virus* cDNA** – in **JOE/HEX channel**.

The results are interpreted according to the crossing (or not-crossing) of the 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.

Data processing

1. In Mx3000P software, 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. Make sure that two fluorescence channels are active (the ***HEX*** and ***FAM*** buttons are activated in the ***Dyes Shown*** field in the lower part of the program's window).
5. Select the ***Threshold fluorescense*** field and make sure that tick marks are put against two fluorescence channels: JOE/HEX and FAM. Check the correctness of the automatically chosen threshold line. Normally, the threshold line is to intersect only with S-shaped 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 raise the threshold level.
6. Select ***Text Report*** point in the ***Area to analyze*** window. Visually ensure that all data is sorted by the name of dyes (the ***Dye*** column). To do this, press once the name of the column (***Dye***).
7. Select ***Export Text Report*** in the ***File*** menu, then select ***Export Text Report to Excel.*** A Microsoft Excel window will open.

Amplification data analysis for the *Rubella virus* cDNA (JOE/HEX channel)

1. Microsoft Excel window will open. In the results grid, ***Ct*** values will appear (column Ct (dR)).
2. For Negative Control of Extraction (C-) – **Negative Control (C-)** – *Ct* values should be absent.
3. For Negative Control of Amplification (NCA) – **RNA-buffer** – *Ct* values should be absent.
4. For the Positive Control of Extraction – **Positive Control cDNA *Rubella virus* / STI (C+)** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin*.
5. For Positive Control of Amplification – **Positive Control *Rubella virus*-rec** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin.*

Amplification data analysis for the IC (FAM channel)

1. Activate the ***FAM*** button in the ***Dyes Shown*** window.
2. Select ***Text Report*** in the ***Area to analyze.*** Open the ***File*** menu, then select ***Export Text Report*** and ***Export Text Report to Excel***. **Ct** values will appear in the Ct column (dR) of the results grid.

3. For Negative Control of Amplification (NCA) – **RNA-buffer** – *Ct* values should be absent.
4. For the Positive Control of Extraction – **Positive Control cDNA *Rubella virus* / STI (C+)** – the *Ct* value should be less than the value specified in the *Important Product Information Bulletin*.
5. For Negative Control of Extraction (C-) – **Negative Control (C-)** – *Ct* values should be less than the value specified in the *Important Product Information Bulletin*..

List of Changes Made in the Guidelines

VER	Location of changes	Essence of changes
11.05.11 RT	Amplification and data analysis using Rotor-Gene 3000/6000 instrument (Corbett Research, Australia). Programming the Rotor-Gene 3000/6000 instrument	In item 5 table 1 was deleted (Amplification program)
	Amplification and data analysis using iCycler iQ/iQ5 instruments (Bio-Rad, USA)	In item 2 table 3 was deleted (Amplification program)
	Amplification and data analysis using Mx3000P, Mx3005P (Stratagene, USA). Manual programming	In item 1 table 5 was deleted (Amplification program)
23.06.11 LA	Cover page	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
01.02.12 VV	Amplification and data analysis using Rotor-Gene 3000/6000 instrument (Corbett Research, Australia) Data analysis of the *Rubella virus* cDNA (JOE/Yellow channel).	The phrase "The *NTC threshold* value can be raised to 20% if a weak signal (Ct) from the Green/FAM channel is detected for the positive control of RT-PCR in the Yellow/JOE channel. The nonspecific signal associated with cross-detection has a lower rise value than the specific signal" was deleted.
31.07.13 ME	Cover page	IVD symbol was added
	Footer	REF R-V24-S(RG,iQ,Mx)-CE-B was deleted
02.07.15 PM	Through the text	Corrections according to the template
27.02.20 PM	Front page	The phrase "Not for use in the Russian Federation" was added
01.03.21 KK	—	The name, address and contact information for Authorized representative in the European Community was changed