

GUIDELINES

to **AmpliSens[®] HBV-Monitor-FRT PCR kit**

AmpliSens[®] HCV-Monitor-FRT PCR kit

AmpliSens[®] HDV-Monitor-FRT PCR kit

for quantitative detection of *hepatitis C virus (HCV)* RNA, *hepatitis B virus (HBV)* DNA and *hepatitis D (HDV)* RNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection



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INTENDED USE

The guidelines describe the procedure of using **AmpliSens® HCV-Monitor-FRT**, **AmpliSens® HBV-Monitor-FRT** and **AmpliSens® HDV-Monitor-FRT** PCR kits for quantitative detection of *hepatitis C virus (HCV)* RNA, *hepatitis B virus (HBV)* DNA and *hepatitis D (HDV)* RNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection using the following instruments:

- Rotor-Gene 3000 (two and more channels), Rotor-Gene 6000 (five or six channels) (Corbett Research, Australia);
- iCycler iQ5 (Bio-Rad, USA);
- CFX96 (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/DNA extraction from 100 µl - sample with lysis of sample outside of the instrument (off-board mode)

1. Switch on the NucliSENS easyMAG instrument and prepare it to the RNA/DNA extraction according to the Instruction manual.
2. In the window for input of test samples enter the following parameters:
 - Sample name
 - **Matrix** for RNA/DNA extraction (select **Plasma**)
 - **Volume** – 0.1 ml
 - **Eluate** – 55 µl
 - **Type** – Lysed
 - **Priority** – Normal.
3. Create a new protocol of RNA/DNA extraction and save it. In protocol select **On-board Lysis Buffer Dispensing – no, On-board Lysis Incubation – no**.
4. Relocate sample table into the created protocol.
5. Add **450 µl** of **NucliSens lysis buffer** to the wells of the reagent cartridge intended for RNA/DNA extraction in the NucliSENS easyMAG instrument.
6. Add **100 µl** of test plasma into each well of the reagent cartridge using disposable tips

REF R-V5-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE-B /

with filters and carefully mix by pipetting.

7. For each panel it's necessary to run **two Positive Controls of Extraction (PCE-1 and PCE-2)**. To prepare them, add **90 µl of Negative Control (C-)** and **10 µl of PCE-1** to the well of the reagent cartridge intended for **PCE-1** with the NucliSens lysis buffer and carefully mix by pipetting, similarly, add **90 µl of Negative Control (C-)** and **10 µl of PCE-2** to the well of the reagent cartridge intended for **PCE-2** with the NucliSens lysis buffer and carefully mix by pipetting.
8. For each panel it's necessary to run the **Negative Control of Extraction (C-)**. To prepare it, add **100 µl of Negative Control** to the wells of the reagent cartridge intended for **C-** with the NucliSens lysis buffer and carefully mix by pipetting.
9. Incubate the reagent cartridge for 10 min at room temperature to ensure lysis.
10. Mix in a new sterile 1.5-ml tube NucliSens magnetic silica and the appropriate Internal Control by using disposable tips with filters (see table 1).

Table 1

Quantity of samples for RNA/DNA extraction	Quantity of magnetic silica NucliSens, µl	Quantity of Internal Control, µl
1	10	10
8	90	90
16	170	170
24 (complete load instrument)	250 (extra for 25 samples)	250

11. Add **20 µl of prepared mixture of NucliSens magnetic silica and Internal Control** to each well of the reagent cartridge. Carefully mix the contents of each well using a pipette with 1000 µl disposable tips with filters.
12. Place the reagent cartridge with samples into the instrument, insert aspiration tip sets and start the RNA/DNA extraction program with lysis of samples by selecting the **off board** mode.
13. After the extraction procedure is completed, remove the reagent cartridge from the instrument and carry out the RT-PCR/PCR not later than 30 min after the completion of RNA/DNA extraction.

For long storage of the reagent, the supernatant must be transferred to a sterile tube and kept at a temperature range from minus 24 °C to minus 16 °C for no longer than a month or at a temperature range no lower than minus 68 °C for no longer than one year.

Variant 2

RNA/DNA extraction from 0.1 – 1 ml sample with automated lysis of sample in the instrument (on-board mode)

1. Place the vial with the NucliSens lysis buffer into the instrument.
2. Add 100 µl or 1 ml of test plasma using disposable tips with filters to each wells of the reagent cartridge intended for RNA/DNA extraction in the NucliSENS easyMAG instrument.
3. For each panel it's necessary to run two **Positive Controls of Extraction (PCE-1 and PCE-2)**. To prepare them, add **90 µl of Negative Control (C–)** and **10 µl of PCE-1** to the well of the reagent cartridge intended for **PCE-1**, similarly, add **90 µl of Negative Control (C–)** and **10 µl of PCE-2** to the well of the reagent cartridge intended for **PCE-2**.
4. For each panel it's necessary to run the **Negative Control of Extraction (C–)**. To prepare it, add **100 µl of Negative Control (C–)** to the wells of the reagent cartridge intended for **C–**.
5. Switch on the NucliSENS easyMAG instrument and prepare it for the RNA/DNA extraction according to the Instruction manual.
6. In the window for input of test samples enter the following parameters:
 - Sample name
 - **Matrix** for RNA/DNA extraction (select **Plasma**)
 - **Volume** – **100 µl** or **1 ml**, depending on the sample volume being used
 - **Eluate** – **55 µl**
 - **Type** – **Primary**
 - **Priority** – **Normal**.
7. Create a new protocol of RNA/DNA extraction and save it. In the protocol, select **On-board Lysis Buffer Dispensing - yes, On-board Lysis Incubation - yes**.
8. Relocate programmed sample to created protocol.
9. Place the reagent cartridge with samples into the instrument, insert aspiration tip sets, and run the RNA/DNA extraction with sample lysis in the instrument (**on board** mode).
10. Wait for the NucliSENS easyMAG instrument stop working in **Instrument State-Idle** position (near 15 min).
11. Mix in a new sterile 1.5-ml tube NucliSens magnetic silica and appropriate Internal Control by using disposable tips with filters (see table 1).
12. Open the lid of the instrument and add **20 µl of prepared mixture of NucliSens**

REF R-V5-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE-B /

magnetic silica and Internal Control to each well of the reagent cartridge. Carefully mix the contents of each well by using a pipette with 1000 µl disposable tips with filter.

13. Continue the RNA/DNA extraction program.

14. After the RNA/DNA extraction procedure is completed, remove the reagent cartridge from the instrument and carry out the RT-PCR/PCR not later than 30 min after completion of RNA/DNA extraction.

When NucliSENS easyMAG instrument is used, the reagent kit allows working with sample volumes 0.1 or 1 ml.

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

When working with Rotor-Gene 3000 one should use the Rotor-Gene version 6.1 and higher software and the Rotor-Gene 6000 versions 1.7 (build 67) software or higher for Rotor-Gene 6000 instrument.

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 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, run the Rotor-Gene software.
2. Insert the tubes or strips into the rotor of the Rotor-Gene 3000/6000/Q 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.

The first well must be filled with any test tube from this experiment except for an empty one. If the rotor is loaded with tubes containing reagents from different
NOTE: PCR kits, then well No. 1 must contain the tube with the largest number of fluorophores. For example, in simultaneous analysis of *HCV*-genotyping and *HDV* detection, the tubes for *HCV*-genotyping should be placed in the rotor first.

3. Program the instrument according to the Instruction Manual given by the manufacturer of the instrument.

Programming the Rotor-Gene 3000/6000 instrument

1. Click the **New** button in the program main menu.
2. In the newly opened window select **Advanced** and click **Dual Labeled Probe/Hydrolysis probes**. Activate the **New** button.
3. Select **36-Well Rotor** (or **72-Well Rotor**) and tick the boxes next to **No Domed 0.2 ml Tubes/Locking ring attached**. Click **Next**.
4. In the newly opened window set the **Reaction volume** to **50 µl**. Set the operator's name. Click **Next**.
5. In the newly opened window click the **Edit profile** button and program the amplification process for the rotor-type instruments (see Table 1).

Table 1

AmpliSens-2 RG program for rotor-type instruments

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold 1	50	15 min	–	1
Hold 2	95	15 min	–	1
Cycling 1	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	–	

NOTE: Any combination of the tests can be performed in one instrument simultaneously with the use of AmpliSens-2 RG program (for example: simultaneously with *HBV* detection, *HCV* genotyping and other tests). If only the tests for *HBV* DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be deleted from the program for time saving.

Note – The ROX/Orange and Cy5/Red channels are enabled when required if the “multiprime” format tests are performed.

6. Click **OK**.
7. Click the **Calibrate/Gain Optimisation** button in the **New Run Wizard** window:
 - perform the fluorescence detection in FAM/Green, JOE/Yellow, ROX/Orange and Cy5/Red channels (activate the **Calibrate Acquiring/Optimise Acquiring** button);
 - perform the calibration before the first detection (activate the **Perform Calibration Before 1st Acquisition/Perform Optimisation Before 1st Acquisition** button);

- to set channels calibration for all fluorophores, indicate **5** in the **Min Reading** box and **10** in the **Max Reading** box (activate **Edit...**, the window **Auto gain calibration channel settings**). Click **Close**.
- 8. Click **Next**. Start the amplification program by activating the **Start Run** button, and name the experiment.
- 9. Name the experiment and save it to the disk (results of the run will be automatically saved in this file).
- 10. Enter the data into the samples table (opens automatically after the amplification has started). Indicate the names/numbers of the test samples in the **Name** box. Set the type **None** for the cells matching with the corresponding empty tubes.

NOTE: Select **Standard** for DNA calibrators PIC1 and PIC2. Enter the values specified in the *Important Product Information Bulletin* enclosed in the PCR kit of the given lot in the **Given conc.** column.

NOTE: Samples indicated as None will not be analysed.

Data analysis for HCV, HBV or HDV cDNA/DNA (JOE/Yellow channel)

1. Activate the **Analysis** button in the menu, select the mode of the analysis **Quantitation**, activate the buttons **Cycling A. JOE/Cycling A. Yellow, Show**.
2. Cancel the automatic choice of the threshold line level by activating the **Threshold** button.
3. Activate the **Dynamic tube** and **Slope Correct** buttons in the main window menu (**Quantitation analysis**).
4. In the **CT Calculation** menu (in the right part of the window) indicate the threshold level **0.03** in the **Threshold** box.
5. Choose the parameter **More Settings/Outlier Removal** and set **10 %** for the values of negative samples threshold (**NTC threshold**).
6. In the results grid (the **Quant. Results** window) one will be able to see the *Ct* values.

Data analysis for the IC (FAM/Green channel)

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

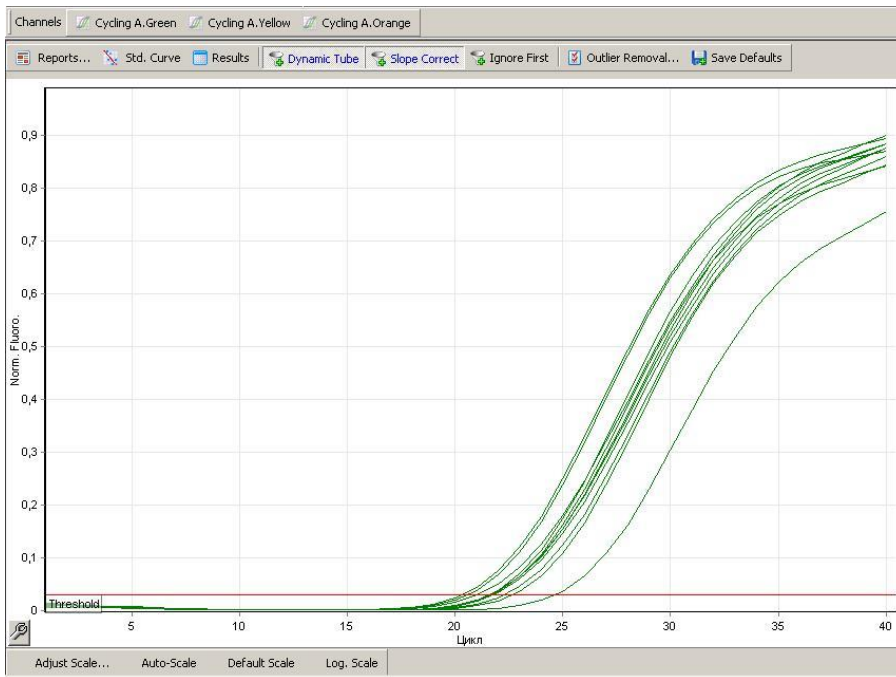
REF R-V5-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE,
REF R-V1-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE-B /

0.03 in the **Threshold** box.

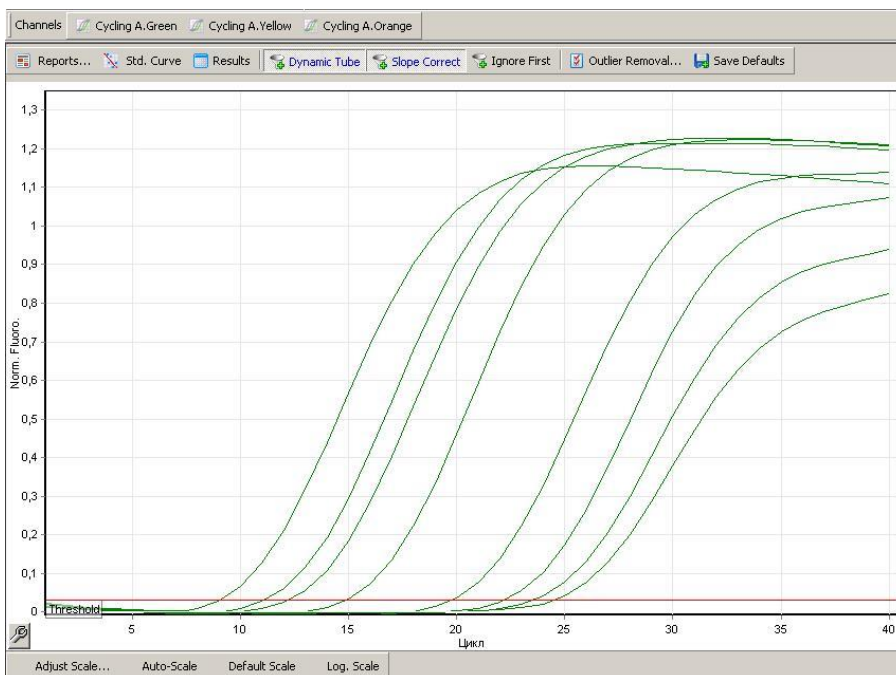
5. Choose the parameter **More Settings/Outlier Removal** and set **10 %** for the value of negative samples threshold (**NTC threshold**).
6. In the results grid (the **Quant. Results** window) one will be able to see the **Ct** values for **IC**.

Example of obtained results

Data in the FAM channel – IC:



Data in the JOE channel – samples, containing the specific target:



REF R-V5-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE,
REF R-V1-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE-B /

AMPLIFICATION AND DATA ANALYSIS USING iCycler iQ5 INSTRUMENT (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 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. Select the **Create new** button in the **Workshop** module.
2. Set the amplification program (see the table below).

Table 2

AmpliSens-2 iQ program for plate-type instruments

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
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	30 s	FAM, HEX, ROX, Cy5	
	72	15 s	–	

NOTE: Any combination of the tests can be performed in one instrument simultaneously with the use of AmpliSens-2 iQ program (for example: simultaneously with *HBV* detection, *HCV* genotyping and other tests). If only the tests for *HBV* DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be deleted from the program for time saving.

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

4. Name the new protocol and save it.
5. Create **Plate Setup** (set the order of the tubes in the reaction chamber).
6. Set all biological samples as **Unknown** in the open window. Set fluorescence detection in **FAM** and **HEX** channels for all samples.

NOTE: Select **Std (Standart)** type for DNA calibrators PIC1 and PIC2. In the **Quantity** column enter the values specified in the *Important Product Information Bulletin* enclosed to the PCR kit for the given lot.

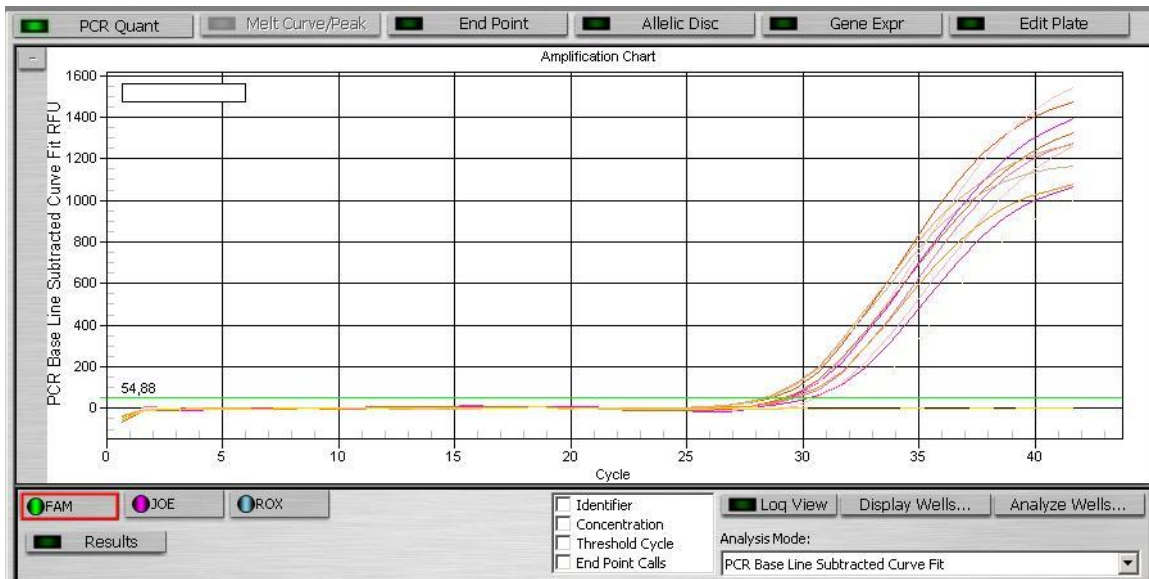
7. Set **Sample volume** – 50 µl. Set **Seal Type** and **Vessel Type**. Click **Save**. Make sure that plastic consumables are the same as those used for instrument calibration.
8. Select **Run**. In the open window select **Use Persistent Well Factors** and click **Begin Run** button. Save the test.

Data analysis:

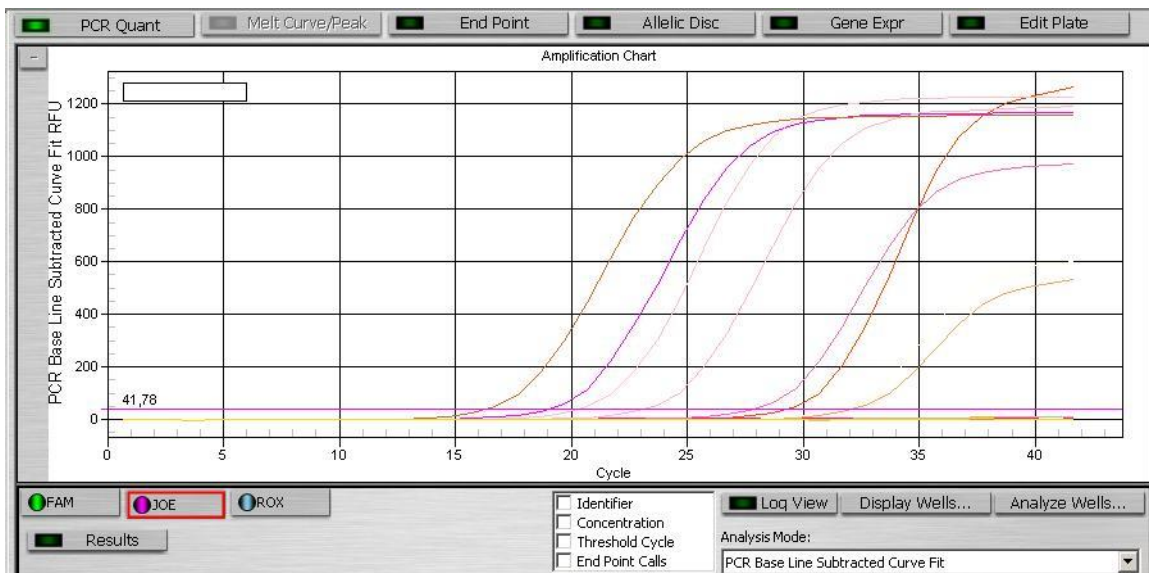
1. Start the program and open the saved file: click **Data file** in the **Workshop** module and select the data file. Proceed to the **Data Analysis** mode.
2. Observe data separately for each channel.
3. Ensure that automatic setting the threshold level is correct for all channels. Normally, the threshold line should intercept only sigmoid curves of signal accumulation of positive samples and controls and should not intercept the base line; otherwise, the threshold level should be raised. To do this, click the **Log View** button and set the threshold (with the left mouse button) at a level where fluorescence curves are linear and do not cross the curves of negative samples.
4. Select the **Results** button (below the buttons of fluorophores) for analysis of results.

Example of obtained results

Data in FAM channel – IC:



Data in HEX channel –samples, containing the specific target:



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

Programming the thermocycler

1. Switch the instrument on, start the program Mx3000P/Mx3005P.
2. Select **Quantitative PCR (Multiple Standards)** in the **New Experiment Options** window and check **Turn lamp on for warm-up** box.

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

3. Insert the tubes into the instrument, lock the fixing arm and the door of the instrument.
4. Select **Optics Configuration** in the **Options** menu and in the **Dye Assignment** tab set the FAM parameter next to the **FAM filter set**, set the JOE parameter next to the **HEX/JOE filter set**, set the ROX parameter next to the **ROX filter set** and the Cy5 parameter next to the **Cy5 filter set** item.
5. Set the fluorescence detection parameters in the **Plate Setup** menu. To do this, select all the cells with the test tubes and mark them as **Unknown** in the **Well type** field.

NOTE: Select **Standard** for DNA calibrators PIC1 and PIC2. Enter the values specified in the *Important Product Information Bulletin* enclosed to the PCR kit of the required lot in the **Standard quantity** column.

6. Select FAM, JOE, ROX, and Cy5 fluorophores in the **Collect fluorescence data** option.
7. Enter the names of all the test samples in the **Well Information** window.
8. In the **Plate Setup** menu, select all cells with test tubes or strips and select **Unknown** and **Collect fluorescence data** in the **Well type** drop-down window. Activate FAM, JOE, ROX, and Cy5 fluorophores.
9. Select the **Thermal Profile Setup** menu and set the amplification program (see table 3).

AmpliSens-2 iQ program for plate-type instruments

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
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	30 s	FAM, JOE/HEX, ROX, Cy5	
	72	15 s	–	

NOTE: Any combination of the tests can be performed in one instrument simultaneously with the use of AmpliSens-2 iQ program (for example: simultaneously with *HBV* detection, *HCV* genotyping and other tests). If only the tests for *HBV* DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be deleted from the program for time saving.

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

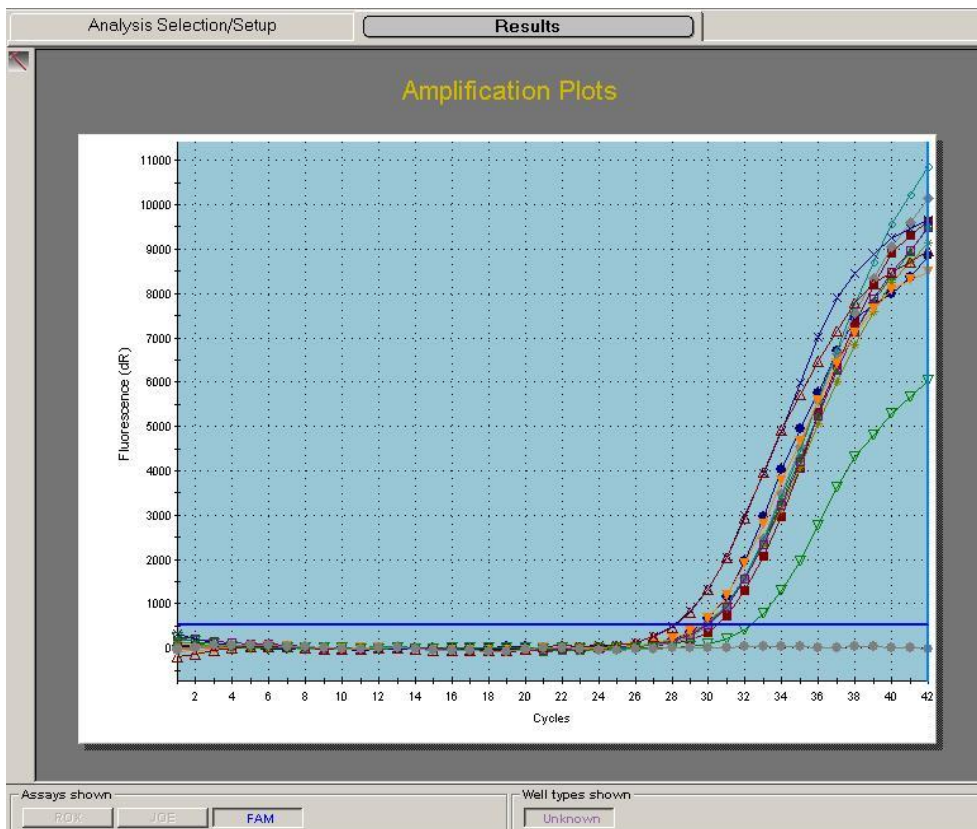
10. Start the amplification by clicking the **Run** and **Start** buttons, then name the experiment.

Data analysis:

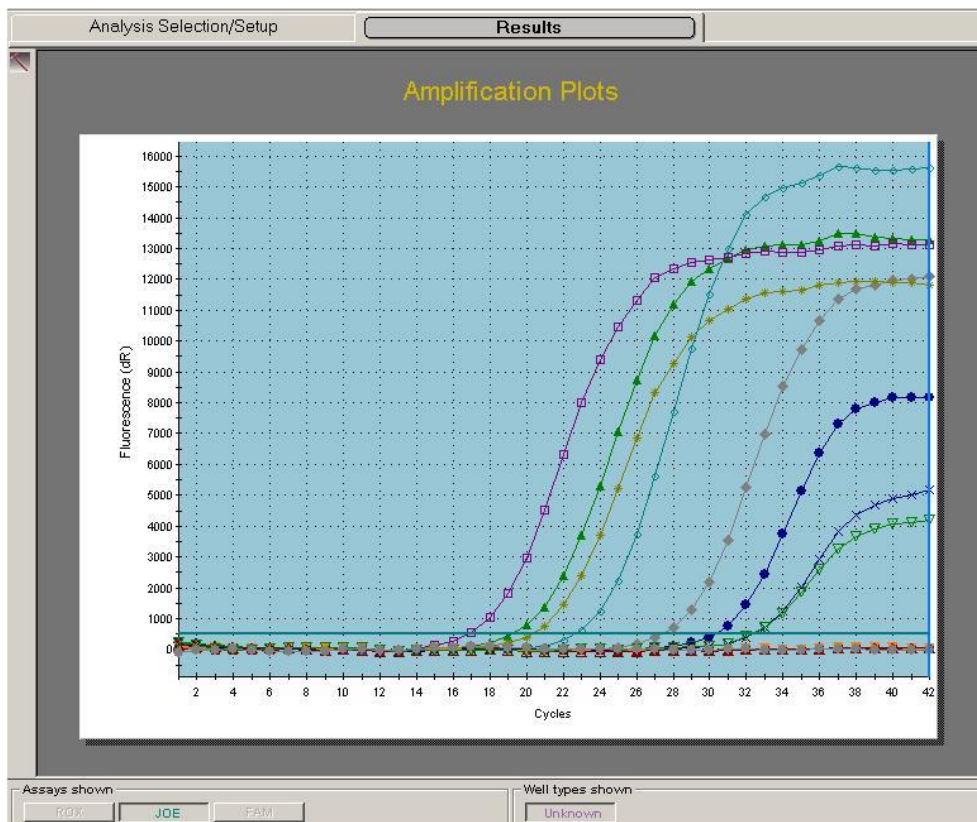
1. Select **Analysis** by clicking the corresponding button of the tool bar.
2. The **Analysis Selection/Setup** tab will open. Make sure that all the test samples are active (the cells corresponding to the samples should be of a different colour).
3. Select the **Results** tab.
4. Ensure that the automatic setting of the threshold is correct for each channel. Normally, the threshold line should intercept only the sigmoid curves of signal accumulation of the positive samples and the controls and should not intercept the base line. If otherwise, the threshold level should be raised. To do this, activate the individual channel display in the **Dyes shown** panel (the fluorescence channels will be displayed separately), check the level of the threshold and change it if necessary.

Examples

Data in FAM channel – IC:



Data in JOE channel –samples that contain the specific target:



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).

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. Do not turn the tubes (strips) upside down while inserting them into the instrument.

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. Select **Create a new Run** (or select **New** and then **Run...** in the **File** menu).
2. 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 the table below). Set **Sample Volume – 50 µl**.

Table 4

AmpliSens-2 program for plate-type instruments

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
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	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.

NOTE: Any combination of the tests can be performed in one instrument simultaneously with the use of AmpliSens-2 program (for example: simultaneously with *HBV* detection, *HCV* genotyping and other tests). If only the tests for *HBV* DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be deleted from the program for time saving.

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

REF R-V5-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE-B /

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.
5. When the required program is entered or edited, click **OK** at the bottom of the window.
6. 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 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.
7. 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.
8. Place the reaction tubes in the wells of the instrument in accordance with the entered plate setup. In the **Start Run** tab click the **Start Run** button then save the file of the experiment.
9. Proceed to the analysis of results after the end of the run.

Analysis of results

Obtained data is interpreted by the real-time PCR instrument software by the crossing of a fluorescence curve with the threshold line set at the specific level (that corresponds to the presence of *Ct* value in the results grid).

The obtained data, that is the curves of accumulation of fluorescence signals in two channels, are analysed with the software of the real-time PCR instrument in use:

- The Internal Control DNA amplification product is detected in the FAM channel;
- The specific target DNA amplification product is detected in the HEX channel.

The fluorescence curves, plate setup, and the results grid with *Ct* values are displayed in the **Quantification** tab. Make sure that the threshold line was set correctly for each channel. Use one of the following variants:

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).


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

Click **Tools** on the toolbar, then **Reports...**, and then save the generated report.

CALCULATION OF CONCENTRATION IN BIOLOGICAL AND CONTROL SAMPLES WITH THE USE OF AmpliSens Soft Monitor FRT SOFTWARE

The procedure of results processing with AmpliSens Soft Monitor FRT software is specified in the "Instruction" worksheet of the software.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
03.07.11 RT	Cover page	The name of Institution was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
29.02.11 lv	Cover page	The AmpliSens® HDV-Monitor-FRT PCR kit was added
	Throughout the text	
	Footer	The catalogue number R-V3-MC(RG,iQ,Mx,Dt)-CE was added
28.09.12 LA	Throughout the text	Section "Procedure for conducting amplification and data analysis with CFX96 (Bio-Rad, USA)" was added
02.11.12 lv	Concentration calculation with the AmpliSens Soft Monitor FRT program	AmpliSens Soft Monitor FRT program version was deleted
23.06.14 ME	Cover page	Intended use of the kit was added
	Intended Use	iCycler iQ was deleted. NucliSENS easyMAG instrument was added
	WORK with the NucliSENS easyMAG AUTOMATED NUCLEIC ACID EXTRACTION SYSTEM	Information about using EM-plus kit was deleted
	Throughout the text	All sections were corrected in accordance to the template
29.01.15 PM	Footer	REF R-V1-MC(RG,iQ,Mx,Dt)-CE-B was added
08.05.15 ME	Text	Clinical material was changed to biological
	Calculation of concentration in biological and control samples with the use of AmpliSens Soft Monitor FRT and AmpliSens Soft Monitor FRT-Q software	Additions about using the AmpliSens Soft Monitor FRT-Q software
08.02.18 DV	Footer	REF R-V5-MC(RG,iQ,Mx,Dt)-CE-B was added
30.04.20 KK	Footer	REF TR-V5-P-M(RG,iQ,Mx,Dt)-CE and REF TR-V1-P-M(RG,iQ,Mx,Dt)-CE were added
29.06.20 EM	Calculation of concentration in biological and control samples with the use of AmpliSens Soft Monitor FRT software	The section was corrected in accordance with the software update
07.10.20 EM	Throughout the text	All the sections were updated according to the template
16.11.20 MM	Footer	REF R-V5-MC(RG,iQ,Mx,Dt)-CE-B and REF R-V1-MC(RG,iQ,Mx,Dt)-CE-B were deleted
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
24.05.21 VA	Footer	REF R-V5-MC(RG,iQ,Mx,Dt)-CE-B, REF R-V1-MC(RG,iQ,Mx,Dt)-CE-B, REF R-V3-MC(RG,iQ,Mx,Dt)-CE-B were added

REF R-V5-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V1-MC(RG,iQ,Mx,Dt)-CE-B, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE-B /