RUO

For Professional Use Only

GUIDELINES

to AmpliSens® *HCV*-FRT PCR kit

AmpliSens® *HDV*-FRT PCR kit

AmpliSens® *HBV*-FRT PCR kit

AmpliSens® *HGV*-FRT PCR kit

AmpliSens®

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TABLE OF CONTENTS

INTENDED USE

The guidelines describe the procedure of using **AmpliSens® *HCV*-FRT, AmpliSens® *HDV*-FRT, AmpliSens® *HBV*-FRT, AmpliSens® *HGV*-FRT** PCR kits for qualitative detection and quantification of *hepatitis C virus* (*HCV)* RNA, *hepatitis D virus* (*HDV)* RNA, *hepatitis G virus* (*HGV)* RNA and *hepatitis B virus* (*HBV)* DNA in the biological material (blood plasma) 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),
- Rotor-Gene Q (QIAGEN, Germany),
- iCycler iQ5 (Bio-Rad, USA),
- CFX96 (Bio-Rad, USA),
- Mx3000P (Stratagene, USA),

and also in association with NucliSENS easyMAG automated nucleic acid extraction system (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 by using disposable tips with filters and carefully mix by pipetting.
7. For each panel it's necessary to run the **Positive Control of Extraction (PCE).** To prepare it, add **90 µl of Negative Control (C–)** and **10 µl** of appropriate **Positive Control** to the wells of the reagent cartridge intended for PCE 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 appropriate Internal Control by using disposable tips with filters (see table 1).

NOTE: When extracting sample to carry out several analyses (simultaneous extraction of nucleic acids for detection of *HDV* RNA, *HCV* RNA, *HGV* RNA, *HBV* DNA, and *HIV* RNA as well as *HCV*-genotyping can be done), add all required IC preparations (by analogy).

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 by 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.

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. For method sensitivity increasing test plasma volume can be varied from 0.1 to 1 ml. Sample lysis is carried out in the NucliSENS easyMAG instrument in automatic mode. The volume of NucliSens lysis buffer is increased to 2 ml. In this case, it's necessary to add 0.1 to 1 ml of test plasma by 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 is necessary to run the **Positive Control of Extraction (PCE).** To prepare it, add **90 µl of Negative Control (C–)** and **10 µl** of appropriate **Positive Control** to the wells of the reagent cartridge intended for PCE.
4. 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–.
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*** – from 100 µl to 1 ml
   - ***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).

NOTE: When extracting sample to carry out several analyses (simultaneous extraction of nucleic acids for detection of *HDV* RNA, *HCV* RNA, *HGV* RNA, *HBV* DNA, and *HIV* RNA as well as *HCV*-genotyping can be done), add all required IC preparations (by analogy).

12. Open the lid of the instrument and 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 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 from 0.1 to 1 ml.

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

When working with the Rotor-Gene 3000 instrument, use the Rotor-Gene version 6 software. When working with the Rotor-Gene 6000/Q instrument, use the Rotor-Gene 6000 versions 1.7 (build 67) software or higher

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 pretreatment reaction mixture preparation stages according to instruction manual. It is recommended to use 0.2-ml clear tubes with flat caps (detection through the bottom of the tube) or 0.1-ml tubes.

Programming the Rotor-Gene 3000/6000/Q instrument

1. Switch on the instrument.
2. Place tubes into the thermocycler rotor ensuring that the first tube appears in well no. 1, place the rotor into the instrument and close the lid (cells are numbered, these numbers are used for following programming of sample placing into the thermocycler).

NOTE: The well no. 1 must be filled by test tube from the current experiment. If one rotor contains tubes with reagents from different PCR kits the well no 1 should be filled by the tube with the most quantity of fluorophores. For example, during, simultaneous *HCV*-genotyping and *HDV* detection analysis, tubes with reagents for *HCV*-genotyping should be placed first into rotor.

3. Press the ***New*** button in the main menu program.
4. In the ***New Run*** window select ***Advanced*** menu and ***Dual Labeled Probe/Hydrolysis probes***. Activate the ***New*** button.

5. Select ***36-Well Rotor*** (or ***72-Well Rotor***) and ***No Domed 0.2 ml Tubes***/***Locking ring attached***. Click the ***Next*** button.
6. ***Reaction volume*** is **25 µl**.
7. Activate function ***15 µl oil layer volume***. Click the ***Next*** button.
8. Select the ***Edit profile*** button.
9. Set the amplification program. Select ***OK***.

Table 2

AmpliSens-2 RG program for rotor-type instruments

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycles***
Hold	50	15 min	–	1
Hold	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	–	

NOTE: Any combination of the tests can be performed in one instrument simultaneously with the use of the unified amplification program (for example, with the tests for *HDV*, *HCV*-genotyping). If only *HBV* DNA is analyzed, the first step (50 °C, 15 min) can be omitted.

NOTE: Channels ROX/Orange and Cy5/Red are switched on when necessary (only in MULTIPRIME assays).

10. In the ***New Run Wizard*** window select the ***Calibrate***/**Gain Optimisation**. For calibration in FAM/Green, JOE/Yellow, ROX/Orange and Cy5/Red channels select ***Calibrate Acquiring/Optimise Acquiring***. Check the ***Perform Calibration Before 1st Acquisition/Perform Optimisation Before 1st Acquisition***. For all channels **FAM/Green**, **JOE/Yellow, ROX/Orange** and **Cy5/Red** set parameters ***Min Reading*** **5Fl**, ***Max Reading*** **10Fl** (***Edit…*** button**,** ***Auto gain calibration channel settings*** window). Press the ***Close*** button, then click the ***Next*** button.
11. Select the ***Start run*** button for amplification run.
12. Name the experiment and save it to the disk (results of the run will be automatically saved in this file).
13. Set the data in the table of samples (open automatically after thermocycling process start). Indicate the names/numbers of test samples in the column ***Name***. For empty wells indicate ***None***.

NOTE: Samples with name ***None*** will not be analyzed.

Data analysis

Data analysis of the *HCV*, *HDV, HGV* or *HBV* cDNA/DNA (JOE/Yellow channel).

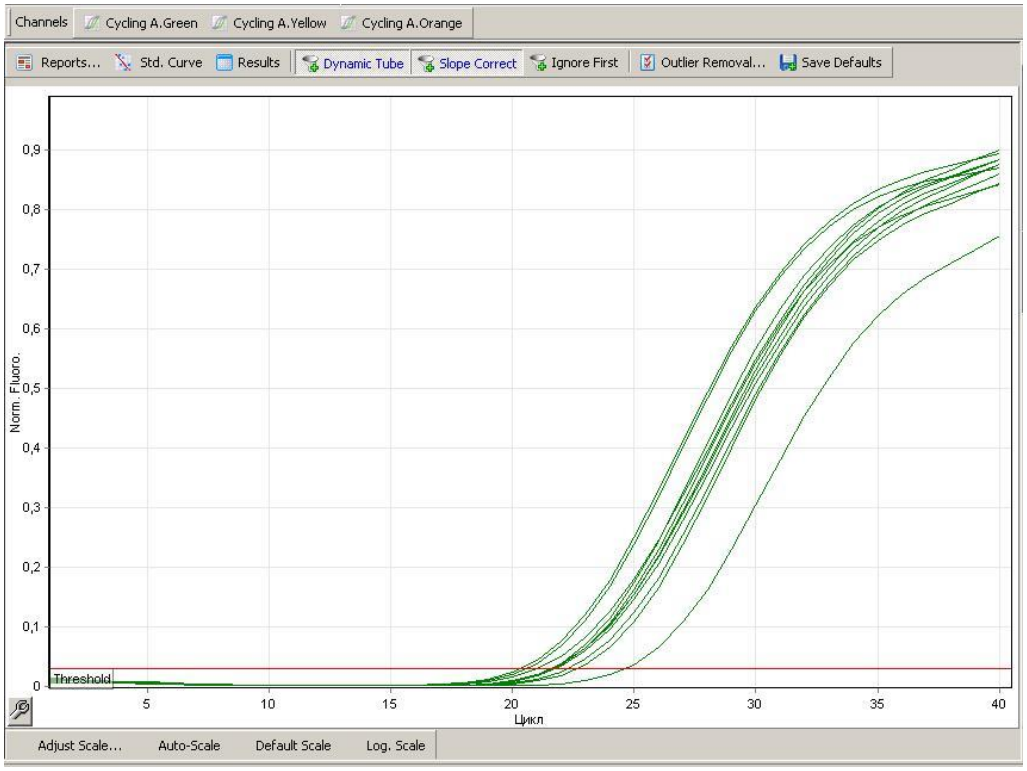
1. Activate the ***Analysis*** button then select ***Quantitation*** button and activate the button ***Cycling A. JOE/Cycling A. Yellow***, and ***Show***.
2. Cancel ***Threshold*** automatic choice.
3. Select the ***Dynamic tube*** and ***Slope Correct*** buttons in the main window menu (set by default).
4. In ***CT Calculation*** menu set ***Threshold* = 0.03.**
5. In the main *window* menu ***More Settings/Outlier Removal*** set ***NTC Threshold*** value 10 %.
6. In the results grid (***Quant. Results*** window) the *Ct* (cycle threshold) values in each test sample will appear.

Data analysis of the IC (FAM/Green channel).

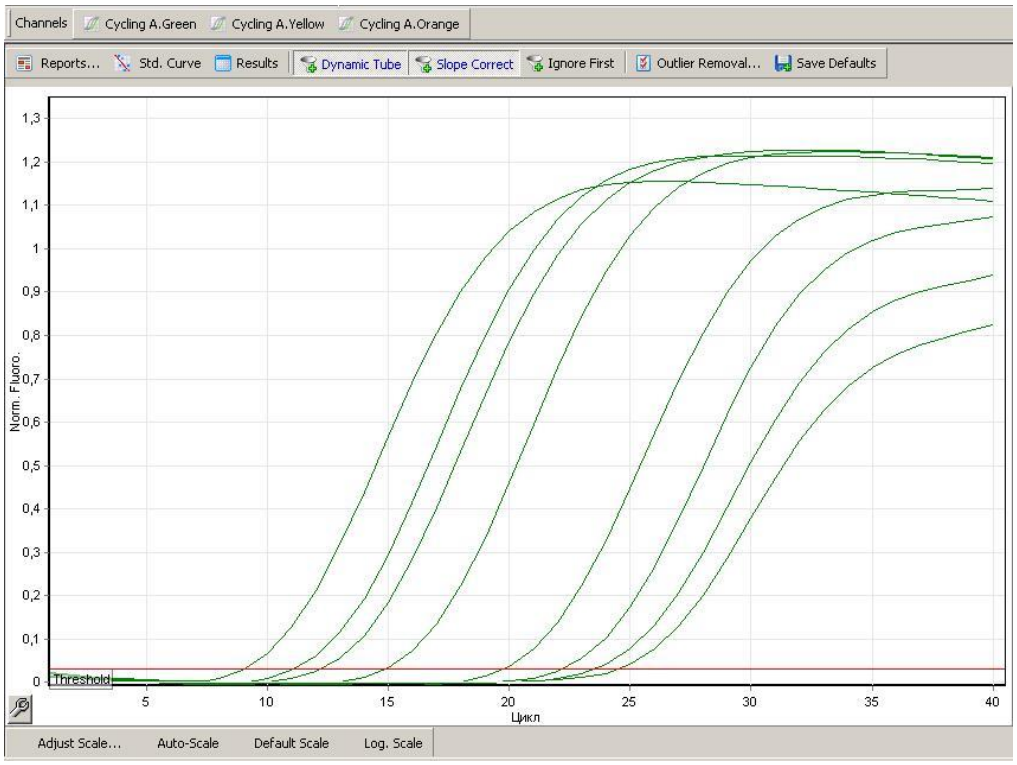
1. Activate the ***Analysis*** button then select ***Quantitation*** button and activate the button ***Cycling A. FAM/Cycling A. Green***.
2. Cancel ***Threshold*** automatic choice.
3. Select the ***Dynamic tube*** and ***Slope Correct*** buttons in the main window menu (set by default).
4. In ***CT Calculation*** menu set ***Threshold* = 0.03.**
5. In the main window menu ***More Settings/Outlier Removal*** set NTC threshold value 10 %.
6. In the results grid (***Quant. Results*** window) the *Ct* (cycle threshold) values for IC in each studying sample will appear.

Example

Data in FAM/Green channel – IC:



Data in JOE/Yellow channel – sample:



AMPLIFICATION AND DATA ANALYSIS USING iCycler iQ5 (BioRad, USA)

Carry out the pretreatment reaction mixture preparation stages according to instruction manual. When working with the iQ5 instrument, it is recommended to use 0.2-ml clear tubes for PCR with domed caps (detection through the cap of the tube).

1. Switch on the instrument.

NOTE: The lamp should be warmed up for at least 15 min before the experiment starts.

2. Open the software.
3. Place tubes/strips into the module. Do not rotate the strips/plate. Make sure there are no drops left on the cell walls.

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.

4. Select ***Create new*** in ***Workshop*** module.
5. Set the amplification program.

Table 3

AmpliSens-2 iQ program for plate-type instruments

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***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	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 the unified amplification program (for example, with the tests for *HDV*, *HCV*-genotyping). If only *HBV* DNA is analyzed, the first step (50 °C, 15 min) can be omitted.

NOTE: Channels ROX and Cy5 are switched on when necessary (only in MULTIPRIME assays).

6. Name the new protocol and save it.
7. Set the ***Plate Setup*** (set the order of the tubes in the reaction chamber).
8. In the opened window mark all biological samples as ***Unknown*****.** Set fluorescence valuation in **FAM and JOE/HEX** channels.
9. Name the plate setup and save it.
10. Set ***Sample Volume*** – **25 µl**. Set ***Seal Type*** and ***Vessel Type***. Click ***Save***. Make sure that plastic consumables are the same as those used for instrument calibration.

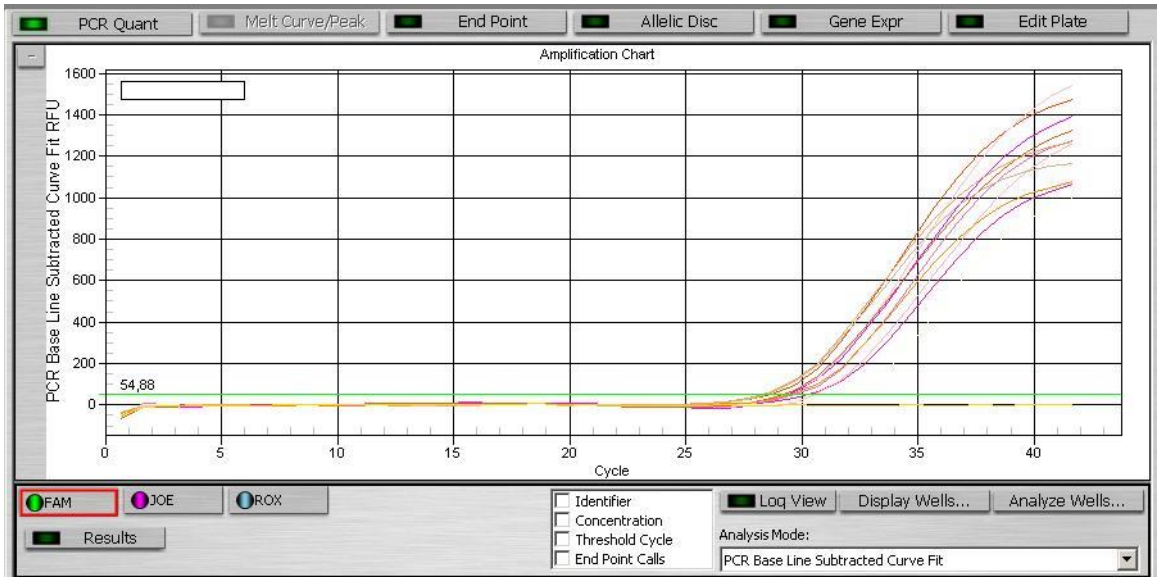
11. Select ***Run***, in open window mark ***Use Persistent Well Factors*** and select ***Begin Run***. Save the test.

Data analysis

1. Start the software and open the saved file: select ***Data file*** in ***Workshop*** module and select the data file. Pass to ***Data Analysis*** mode.
2. Browse data on each channel separately.
3. Ensure that automatic selection of threshold level is correct. Normally, the threshold line should cross only sigmoid curves of signal accumulation of positive samples and controls and not to intersect base lane. If it is not, threshold level should be raised. For this select ***Log View*** and set threshold by left mouse button on level, where fluorescence curves are linear and do not cross curves of negative samples).
4. For result analyses select ***Results***.

Example

Data in FAM channel – IC:



Data in JOE/HEX channel – sample:

AMPLIFICATION AND DATA ANALYSIS USING Mx3000P (Stratagene, USA)

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

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

NOTE: Lamp should be warmed up for at least 15 min before the run starts up.

3. Place experimental tubes into the module and close the lid. Do not rotate the strips/plate. Make sure there are no drops left on the cell walls.
4. Select ***Optics Configuration*** in ***Options*** menu, set ***JOE*** parameters opposite ***HEX/JOE filter set*** item, ***FAM*** parameters opposite ***FAM filter set*** item, ***ROX*** parameters opposite ***ROX filter set*** item, ***Cy5*** parameters opposite ***Cy5 filter set*** item.
5. Set fluorescence detection parameters in ***Plate Setup*** menu. For this:
   - Select all cells with test tubes or stripes (holding **Ctrl** and selecting needed range)
   - Mark all selected cells as ***Unknown*** in ***Well type*** window. Set **FAM**, **JOE, ROX** and **Cy5** in ***Collect fluorescence data*** option. Name each sample by double clicking on each cell (***Well Information*** window).
6. In ***Thermal Profile Setup*** insert set amplification program.

Table 4

AmpliSens-2 iQ program for plate-type instruments

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***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	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 the unified amplification program (for example, with the tests for *HDV*, *HCV*-genotyping). If only *HBV* DNA is analyzed, the first step (50 °C, 15 min) can be omitted.

NOTE: Channels ROX and Cy5 are switched on when necessary (only in MULTIPRIME assays).

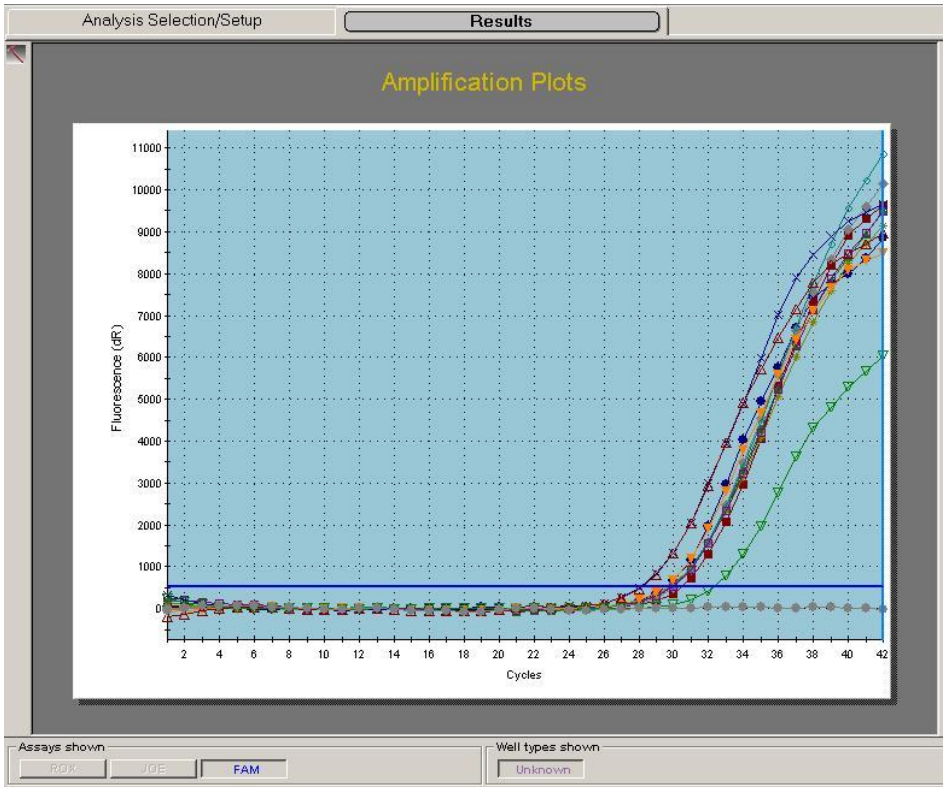
7. Select ***Run*** and ***Start*** and name the experiment file.

Data analysis.

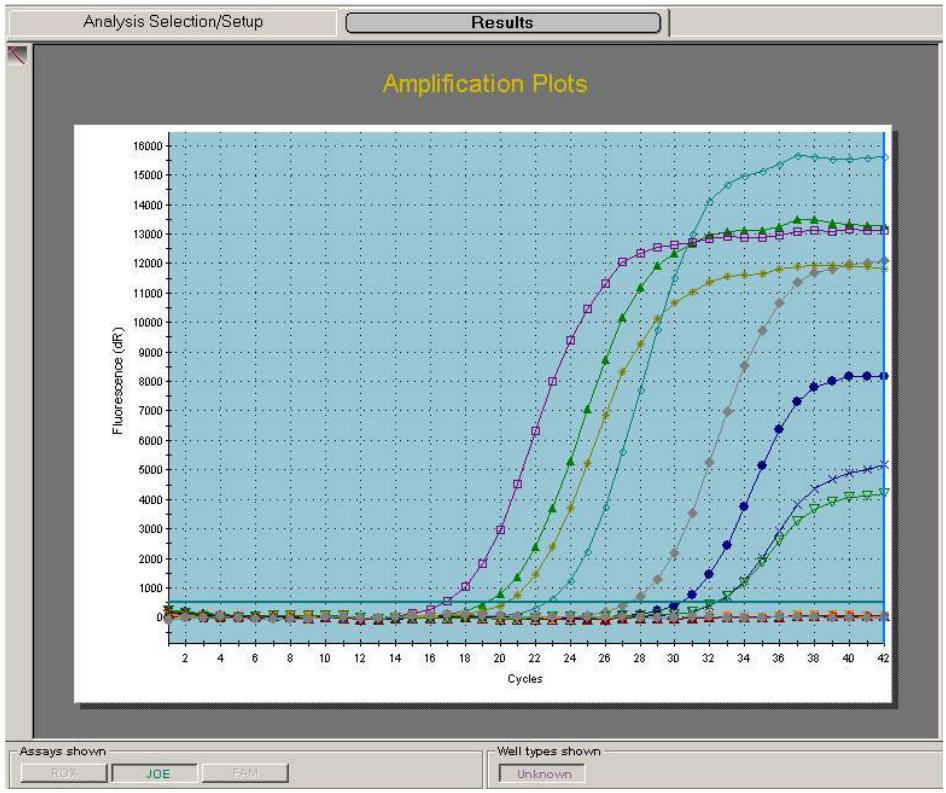
1. In Mx3000P software pass to ***Data Analysis*** mode.
2. Ensure that all samples are active in ***Analysis Selection/Setup*** tab (this cells have another colour).
3. Pass to ***Results*** tab.
4. Check the correctness of threshold line automatic choice for each channel. The threshold line is to cross only with 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, threshold level should be raised. For this activate each channel separately in ***Dyes shown*** panel. Look at the threshold line location and change it if it is necessary.

Example

Data in FAM channel – IC:



Data in JOE/HEX channel – sample:



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

Carry out the pretreatment and reaction mixture preparation stages according to the PCR kit instruction manual. It is recommended that 0.2-ml PCR tubes with optically transparent domed or flat caps are used (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 tubes (strips) upside down while inserting them into the instrument.

Program the instrument in accordance with the Operation Manual provided by the manufacturer.

1. Turn on the instrument and start the ***Bio-Rad CFX Manager*** program.
2. Select ***Create a new Run*** (or select ***New*** and then ***Run…*** in the ***File*** 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 5). Set ***Sample Volume – 25 µl.***

Table 5

AmpliSens-2 program for plate-type instruments

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*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	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 the unified amplification program (for example, with the tests for *HDV*, *HCV*-genotyping). If only *HBV* DNA is analyzed, the first step (50 °C, 15 min) can be omitted.

NOTE: Channels ROX and Cy5 are switched on when necessary (only in MULTIPRIME assays).

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. 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.
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. 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.
8. Proceed to the analysis of results after the end of the run.

Analysis of results

Obtained data are 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).

1. 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.

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 Control 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

2. Click the ***View/Edit Plate*** button on the toolbar and enter sample names in the opened window if required.
3. Click ***Tools*** on the toolbar, then ***Reports…***, and then save the generated report.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
13.07.10	Page footer	Reference numbers were changed from R-V1-Mod(RG,iQ,Mx,Dt); R-V3(RG,iQ,Mx,Dt); R-V5-Mod(RG,iQ,Mx,Dt) to R-V1-Mod(RG,iQ,Mx,Dt)-CE; R-V3(RG,iQ,Mx,Dt)-CE; R-V5-Mod(RG,iQ,Mx,Dt)-CE
15.03.11 LA	Page footer	Reference number was added: R-V1-Mod(RG,iQ,Mx,Dt)-CE-B
06.02.12 VV	Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia) and Rotor-Gene Q (Oiagen, Germany)	Rotor-Gene Q (Qiagen, Germany) was added-
	Text	Information about AmpliSens® *HGV*-FRT PCR kit was added
		Text corrections
		Amplification programs were added
		Section INTENDED USE was added
	Work with the NucliSENS easyMAG automated nucleic acid extraction system	The procedure of extraction was adjusted (text corrections)
	Page footer	Reference number REF R-V2-50-F(RG,iQ,Mx,Dt)-CE was added
26.06.12 LA	Throughout the text	Section “Amplification and data analysis using CFX96 (Bio-Rad, USA)” was added
04.02.14 ME	Intended use	Information about using NucliSENS easyMAG automated nucleic acid extraction system was added. iCycler iQ and Mx3005P was deleted
	Work with the NucliSENS easyMAG automated nucleic acid extraction system, Variant 1	Point 1 about “EM-plus” reagent kit was deleted
		Information about RT-G in points 7 and 8 was deleted
		Information about component A in point 10 and 11 was deleted
		Column “Quantity of component A “EM-plus”, µl” was deleted from the table 1
		Volume of prepared mixture was changed from 30 µl to 20 µl in point 11
	Work with the NucliSENS easyMAG automated nucleic acid extraction system, Variant 2	Information about RT-G in point 1 was deleted
		Information about component A in point 11 and 12 was deleted
		Volume of prepared mixture was changed from 30 µl to 20 µl in point 12
27.01.15 ME	Footer	**REF** R-V3(RG,iQ,Mx,Dt)-CE-B was added

VER	Location of changes	Essence of changes
03.04.15 ME	Text	Corrections according to the template
	Intended use	Types of biological material was added
	Work with the NucliSENS easyMAG automated nucleic acid extraction system	The positive control of extraction preparing was corrected
01.06.15 ME	Title page	The **RUO** symbol was deleted
02.08.17 ME	Footer	**REF** R-V5-Mod(RG,iQ,Mx,Dt)-CE-B was added
16.11.20 KK	Footer	The **REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE-B and **REF** R-V3(RG,iQ,Mx,Dt)-CE-B were deleted
29.12.20 KK	Through the text	The symbol ⚠ was changed to NOTE:
	Cover page	The phrase “Not for use in the Russian Federation” was added
12.02.21 KK	Footer	**REF** R-V5-Mod(RG,iQ,Mx,Dt)-CE-B was deleted
01.06.21 MM	Footer	The **REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE-B was added
02.06.21 KK	Cover page	The phrase “For research use only. Not for diagnostic procedures” was added
04.08.23 BA	Footer	The **REF** R-V3(RG,iQ,Mx,Dt)-CE-B was added