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For Professional Use Only

AmpliSens® *HCV* / *HBV* / *HIV*-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of hepatitis C virus RNA (*HCV*), hepatitis B virus DNA (*HBV*) and human immunodeficiency virus RNA (*HIV*) in the clinical material by using real-time hybridization-fluorescence detection.

This kit can be used for analysis of both individual samples and several plasma samples combined in a minipool. For individual samples, the volume of plasma for nucleic acid extraction is 100, 200 or 1000 µl. For mini-pools, the recommended volume of sample is 1000 µl. The volume of each sample in mini-pool should be no less than 100 µl. The recommended number of plasma samples, combined to mini-pool, is 4–10 with the resulting volume of 1000 µl.

Extraction methods, volumes used for extraction, and the possibility of testing samples combined into mini-pools are specified in Table 1.

Table 1

Extraction method	Volumes of plasma used for RNA/DNA extraction, µl	Possibility of testing plasma mini-pools
NucliSENS easyMAG	100	no
	1000	Up to 10 samples
MAGNO-sorb	200	no
	1000	Up to 10 samples
QIAsymphony Virus/Bacteria Midi Kit	1000	Up to 10 samples
RIBO-sorb	100	no
RIBO-prep	100	no

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Detection of hepatitis C virus RNA (*HCV*), hepatitis B virus DNA (*HBV*) and human immunodeficiency virus type 1 RNA (*HIV*-1) in clinical material by using real-time hybridization-fluorescence detection includes the following stages: (1) RNA/DNA extraction from blood plasma together with Internal Control sample (IC) by means of QIAsymphony SP and NucliSENS easyMAG–automated systems or by means of manual extraction with RIBO-sorb, MAGNO-sorb or RIBO-prep kits; (2) reverse transcription of RNA and amplification of DNA/cDNA by using real-time hybridization-fluorescence detection. In the

channel for the FAM fluorophore the *HCV* cDNA amplification product is detected. In the channel for the JOE fluorophore the *HIV*-1 cDNA amplification product is detected. In the channel for the ROX fluorophore the *HBV* DNA amplification product is detected. In the channel for the Cy5 fluorophore the IC amplification product is detected. In the channel for the Cy5.5 fluorophore the *HIV*-2 cDNA amplification product is detected. The PCR kit variant FRT-4x is intended for the thermocyclers with four fluorescence detection channels. When using PCR kit variant FRT-4x the *HIV*-2 cDNA is not being detected. The Positive Control of Extraction – Positive Control *HCV* / *HBV* / *HIV*-rec – is a complex control for 4 infections; it is detected in the channels for the FAM (*HCV*), JOE (*HIV*-1), ROX (*HBV*), Cy5 (IC) and Cy5.5 (*HIV*-2) fluorophores. The Positive Control of Amplification – Positive Control cDNA *HCV* / *HBV* / *HIV* (C+$_{HCV\ /\ HBV\ /\ HIV}$) – is also a complex control for *HCV*, *HBV*, *HIV*-1, *HIV*-2 and IC; it is detected in corresponding channels.

3. CONTENT

AmpliSens® *HCV / HBV / HIV*-FRT is produced in 3 forms:

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit variant FRT **REF** R-V62(RG,Dt)-CE;

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit variant FRT-4x,

REF R-V50-4x(RG,iQ,Mx,Dt)-CE.

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit variant FRT-4x in bulk[1],

REF R-V50-4x(RG,iQ,Mx,Dt)-CE-B.

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit variant FRT includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
RT-G-mix-2	colorless clear liquid	0.015	4 tubes
RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV*1 / *HIV*2	colorless clear liquid	0.3	4 tubes
RT-PCR-mix-2-FL	colorless clear liquid	0.3	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	4 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.015	4 tubes
Buffer for elution	colorless clear liquid	1.2	4 tubes

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

Positive Control cDNA *HCV* / *HBV* / *HIV* (C+$_{HCV / HBV / HIV}$)	colorless clear liquid	0.1	4 tubes
Negative Control (C–)*	colorless clear liquid	0.5	4 tubes
Positive Control *HCV* / *HBV* / *HIV*-rec**	colorless clear liquid	0.2	4 tubes
Internal Control STI-87-rec (IC)***	colorless clear liquid	0.5	4 tubes

* must be used in the extraction procedure as Negative Control of Extraction.

** must be used in the extraction procedure as Positive Control of Extraction.

*** add 10 µl of Internal Control STI-87-rec during the RNA/DNA extraction procedure directly to the sample/lysis mixture

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit variant FRT is intended for 100 reactions, including controls.

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit variant FRT-4x includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
RT-G-mix-2	colorless clear liquid	0.015	4 tubes
RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV*	colorless clear liquid	0.3	4 tubes
RT-PCR-mix-2-FL	colorless clear liquid	0.3	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	4 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.015	4 tubes
Buffer for elution	colorless clear liquid	1.2	4 tubes
Positive Control cDNA *HCV* / *HBV* / *HIV* (C+$_{HCV / HBV / HIV}$)	colorless clear liquid	0.1	4 tubes
Negative Control (C–)*	colorless clear liquid	0.5	4 tubes
Positive Control *HCV* / *HBV* / *HIV*-rec**	colorless clear liquid	0.2	4 tubes
Internal Control STI-87-rec (IC)***	colorless clear liquid	0.5	4 tubes

* must be used in the extraction procedure as Negative Control of Extraction.

** must be used in the extraction procedure as Positive Control of Extraction.

*** add 10 µl of Internal Control STI-87-rec during the RNA/DNA extraction procedure directly to the sample/lysis mixture

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit variant FRT-4x is intended for 100 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA/RNA extraction kit or RNA/DNA extraction automatic station.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- **For PCR kit variant FRT:** Real-time instruments (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); CFX96 (Bio-Rad, USA) or equivalent) with possibility of simultaneous detection in five channels (corresponding to FAM, JOE, ROX, Cy5, Cy 5.5 fluorescent dyes).

  For PCR kit variant FRT-4x: Real-time instruments (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); CFX96 (Bio-Rad, USA) or equivalent) with possibility of simultaneous detection in four or more channels (corresponding to FAM, JOE, ROX, Cy5 fluorescent dyes).
- Disposable polypropylene tubes for PCR (0.1- or 0.2-ml)
  a) 0.2-ml PCR tubes or strips with domed caps (for example, Axygen, USA) if a plate-type instrument is used;
  b) 0.2-ml PCR tubes (flat caps, nonstriped) (for example, Axygen, USA) for 36-well rotor or 0.1-ml tubes (Corbett Research, Australia) for 72-well rotor if a rotor-type instrument is used.
- Refrigerator at the temperature from 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.

- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

Analysis is performed with peripheral blood plasma. Blood is obtained after overnight fasting to tube with EDTA solution as anticoagulant. Turn the closed tube several times. Plasma should be transferred to a new tube within 6 h after bleeding. This is performed by centrifuging the tube with blood at 800–1600 g for 20 min. Plasma can be stored for no more than 5 days at 2–8°C and for a long time at ≤ –68°C.

In some cases, blood serum can be used. The analytical sensitivity of the reagent kit for this material is the same, but the clinical sensitivity may greatly decrease because of sedimentation of viral particles as a result of retraction of the clot. Store blood serum for no

more than 5 days at 2–8 °C and for a long time at ≤ –68°C.

This kit can be used for analyzing both individual samples and several plasma samples combined into a mini-pool. The recommended number of plasma samples for pooling is 4–10 (the resulting volume, 1000 µl).

7. WORKING CONDITIONS

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA/DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- RIBO-sorb, REF K2-1-Et-100-CE;
- RIBO-prep, REF K2-9-Et-100-CE;
- MAGNO-sorb, REF K2-16-1000-CE.

QIAsymphony SP and NucliSENS easyMAG automated system can be used.

If using RIBO-prep kit, extract the RNA/DNA according to the manufacturer's protocol taking into account next additions and improvements:

- It is allowed to mix the **Solution for lysis** and **Internal Control STI-87-rec (IC)** in a separate sterile vial (300 µl of **Solution for lysis** and 10 µl of **Internal Control STI-87-rec (IC)** per sample) and then transfer 300 µl of mixture to each prepared 1.5-ml tube to simplify the extraction procedure in case of great quantity of samples.
- To prepare the Positive Control of extraction, **PCE**, add **100 µl** of the **Positive Control *HCV* / *HBV* / *HIV*-rec** sample to a tube containing **Solution for Lysis.**

If using RIBO-sorb kit, extract the RNA/DNA according to the manufacturer's protocol taking into account next additions and improvements:

- Add **10 µl** of **Internal Control STI-87-rec (IC)** to each tube and then add **450 µl** of **Lysis Solution**.
- It is allowed to mix the **Lysis Solution** and **Internal Control STI-87-rec (IC)** in a separate sterile vial (450 µl of **Lysis Solution** and 10 µl of **Internal Control STI-87-rec (IC)** per sample) and then transfer 450 µl of mixture to each prepared 1.5-ml tube to simplify the extraction procedure in case of great quantity of samples.
- To prepare the Positive Control of extraction, **PCE**, add **100 µl** of the **Positive Control *HCV* / *HBV* / *HIV*-rec** sample to a tube containing **Lysis Solution.**

If using the MAGNO-sorb kit extract the RNA/DNA according to the manufacturer's protocol taking into account next additions and improvements:

- In case of RNA/DNA extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control STI-87-rec (IC)** required for 24-tube panel is

0.28 ml. In case of other panels and RNA/DNA extraction from blood plasma sample of 200 µl see MAGNO-sorb instruction manual.

- To prepare the Positive Control of extraction, **PCE**, add **100 µl** of the **Positive Control *HCV* / *HBV* / *HIV*-rec** sample to a tube containing **Lysis Solution MAGNO-sorb**.
- To prepare the Negative Control of extraction, **C–**, add **100 µl** of the **Negative Control (C–)** sample to a tube containing **Lysis Solution MAGNO-sorb**.
- The volume of **Buffer for elution** required for extraction from both 1000 and 200 µl of blood plasma samples is **70 µl**.

If using the QIAsymphony SP automated system extract the RNA/DNA according to the Guiedelines [2].

8.2. Preparing reverse transcription and PCR

Total reaction volume is **50 µl**, the volume of RNA/DNA sample is **30 µl**.

RNase-free and Dnase-free plastic ware should be used only.

8.2.1 Preparing tubes for RT-PCR

Variant FRT

Tubes type depends on the thermocycler used. Use filter tips for transferring the reagents and samples into the tubes.

All components of the reaction mixture should be mixed immediately before use.

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Sediment the drops from the tubes caps.
2. Take the required number of PCR-tubes for clinical and control samples (2 controls of the extraction stage and 2 controls of amplification stage). The type of tubes, strips or plates depends on the real-time PCR instrument used for analysis.
3. Prepare the reaction mixture (see Table 1) for the required number of reactions including the analysis of test and control samples.

Table 1

Reaction mixture preparation

		Reagent volume per specified number of PCR reactions with extra volume				
Reagent volume per 1 reaction, µl		**10.00**	**10.00**	**1.00**	**0.50**	**0.50**
Number of clinical samples	**Number of PCR reactions[2]**	**RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV*1 / *HIV*2**	**RT-PCR-mix-2-FL**	**Polymerase (TaqF)**	**RT-G-mix-2**	**TM-Revertase (MMlv)**
6	**10**	**110**	**110**	**11**	**5**	**5**
8	**12**	**130**	**130**	**13**	**6**	**6**
10	**14**	**150**	**150**	**15**	**7**	**7**
12	**16**	**170**	**170**	**17**	**8**	**8**
14	**18**	**190**	**190**	**19**	**9**	**9**
16	**20**	**210**	**210**	**21**	**10**	**10**
18	**22**	**230**	**230**	**23**	**11**	**11**
20	**24**	**250**	**250**	**25**	**12**	**12**
22[3]	**26**	**Whole tube content**	**Whole tube content**	**Whole tube content**	**15**	**Whole tube content**

4. For analysis of 22 clinical samples (24 samples after extraction procedure) the following method is recommended: transfer the whole volume of **RT-PCR-mix-2-FL** (0.3 ml), the whole volume of **polymerase (TaqF)** (0.03 ml), the whole volume of **TM-Revertase (MMlv)** (0.015 ml) and the whole volume of **RT-G-mix-2** (0.015 ml) into the tube with **RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV*1 / *HIV*2** (0.3 ml). Thoroughly mix by vortexing and sediment the drops from the tubes caps.
5. Add **20 µl** of prepared reaction mixture into each PCR-tube. Discard the remaining mixture.
6. Using filter tips add **30 µl** of RNA/DNA-sample to reaction mixture. Thoroughly mix the content by pipetting avoiding foaming.
7. Carry out the control amplification reactions**:**

C+ - Add **30 µl** of **Positive Control cDNA *HCV* / *HBV* / *HIV* (C+$_{HCV\ /\ HBV\ /\ HIV}$)** to the tube labeled C+.

NCA - Add **30 µl** of **Buffer for elution** to the tube labeled NCA.

C– - Add **30 µl** of **the sample extracted from the Negative Control (C–)** reagent to the tube labeled C– (Negative control of Extraction).

[2] Number of clinical samples + 2 controls for RNA extraction stage + 2 controls of RT-PCR (N+4, N – number of clinical samples).

[3] Typical extraction from 24 samples using NucliSENS easyMAG automated system or MAGNO-sorb nucleic acids extraction kit.

PCE - Add **30 µl** of **the sample extracted from the Positive Control *HCV* / *HBV* / *HIV*-rec** reagent to the tube labeled PCE (Positive control of Extraction).

Tubes with control samples are to be mixed by pipetting avoiding foaming.

Variant FRT-4x

Tubes type depends on the thermocycler used. Use filter tips for transferring the reagents and samples into the tubes.

All components of the reaction mixture should be mixed immediately before use.

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Sediment the drops from the tubes caps.
2. Take the required number of PCR-tubes for clinical and control samples (2 controls of the extraction stage and 2 controls of amplification stage). The type of tubes, strips and plates depends on the real-time PCR instrument used for analysis.
3. Prepare the reaction mixture (see Table 2) for the required number of reactions including the analysis of test and control samples.

Table 2

Reaction mixture preparation

		Reagent volume per specified number of PCR reactions with extra volume				
Reagent volume for 1 reaction, µl		**10.00**	**10.00**	**1.00**	**0.50**	**0.50**
Number of clinical samples	**Number of PCR reactions[4]**	**RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV***	**RT-PCR-mix-2-FL**	**Polymerase (TaqF)**	**RT-G-mix-2**	**TM-Revertase (MMlv)**
6	**10**	**110**	**110**	**11**	**5**	**5**
8	**12**	**130**	**130**	**13**	**6**	**6**
10	**14**	**150**	**150**	**15**	**7**	**7**
12	**16**	**170**	**170**	**17**	**8**	**8**
14	**18**	**190**	**190**	**19**	**9**	**9**
16	**20**	**210**	**210**	**21**	**10**	**10**
18	**22**	**230**	**230**	**23**	**11**	**11**
20	**24**	**250**	**250**	**25**	**12**	**12**
22[5]	**26**	**Whole tube content**	**Whole tube content**	**Whole tube content**	**15**	**Whole tube content**

[4] Number of clinical samples + 2 controls for RNA extraction stage + 2 controls of RT-PCR (N+4, N – number of clinical samples).

[5] Typical extraction from 24 samples using NucliSENS easyMAG automated system or MAGNO-sorb nucleic acids extraction kit.

4. For analysis of 22 clinical samples (24 samples after extraction procedure) the following method is recommended: transfer the whole volume of **RT-PCR-mix-2-FL** (0.3 ml), the whole volume of **polymerase (TaqF)** (0.03 ml), the whole volume of **TM-Revertase (MMlv)** (0.015 ml) and the whole volume of **RT-G-mix-2** (0.015 ml) into the tube with **RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV*** (0.3 ml). Thoroughly mix by vortexing and sediment the drops from the tubes caps.
5. Add **20 µl** of prepared reaction mixture into each PCR-tube. Discard the remaining mixture.
6. Using filter tips add **30 µl** of RNA/DNA-sample to reaction mixture. Mix the content by pipetting avoiding foaming.
7. Carry out **control amplification reactions:**

C+ - add **30 µl** of **Positive Control cDNA *HCV* / *HBV* / *HIV* (C+$_{HCV\ /\ HBV\ /\ HIV}$)** to the tube labeled C+.

NCA - add **30 µl** of **Buffer for elution** to the tube labeled NCA.

C– - Add **30 µl** of **the sample extracted from the Negative Control (C–)** reagent to the tube labeled C– (Negative control of Extraction).

PCE - Add **30 µl** of **the sample extracted from the Positive Control *HCV* / *HBV* / *HIV*-rec** reagent to the tube labeled PCE (Positive control of Extraction).

Tubes with control samples are to be mixed by pipetting avoiding foaming.

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows (see Tables 3, 4):

Table 3

AmpliSens *HBV* / *HCV* / *HIV* amplification program for rotor-type instruments[6]

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
1	50	20 min	–	1
2	95	15 min	–	1
3	95	20 s	–	4
	46	40 s	–	
4	95	5 s	–	42
	60	40 s	–	
	45	30 s	FAM, JOE, ROX, Cy5, Cy 5.5	

When using PCR kit variant FRT-4x the channel for the Cy 5.5 fluorophore is not used.

Table 4

AmpliSens *HBV* / *HCV* / *HIV* amplification program for plate-type instruments[7]

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
1	50	20 min	–	1
2	95	15 min	–	1
3	95	20 s	–	4
	46	40 s	–	
4	95	5 s	–	42
	60	40 s	–	
	40	40 s	FAM, JOE, ROX, Cy5, Cy 5.5	

When using PCR kit variant FRT-4x the channel for the Cy 5.5 fluorophore is not used.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the plate-type instrument instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

DATA ANALYSIS

The results are interpreted by the real-time PCR instrument software:

[6] For example, Rotor-Gene 3000 and Rotor-Gene 6000 (Corbett Research, Australia).
[7] For example, CFX96 (Bio-Rad, USA).

- *HCV* cDNA amplification product is detected in the channel for the FAM fluorophore.
- *HIV*-1 cDNA amplification product is detected in the channel for the JOE fluorophore.
- *HBV* DNA amplification product is detected in the channel for the ROX fluorophore.
- IC cDNA amplification product is detected in the channel for the Cy5 fluorophore.
- *HIV*-2 cDNA amplification product is detected in the channel for the Cy5.5 fluorophore.

The results are interpreted by the crossing (or not crossing) the threshold line by the fluorescence curve that indicates the presence (or absence) of the threshold *Ct* value in corresponding column in the results table.

The result of amplification is considered *positive* if the fluorescence S-shaped curve (typical for real-time PCR) crosses once the threshold line in the fluorescence reliable growth area; and the threshold value (*Ct* or *Cp*) for respective channel is less than the *Ct* value specified in the *Important Product Information Bulletin*.

The result of amplification is considered *negative* if the fluorescence curve is not S-shaped or does not cross the threshold line (*Ct* or *Cp* values are absent).

Otherwise, the result of amplification is considered *equivocal*.

For details, see Instruction Manuals for appropriate instruments and Guidelines [2].

The sample is considered positive for *HBV* DNA, *HCV* RNA, *HIV*-1 RNA and *HIV*-2 RNA if the *Ct* value defined in respective channel is less than the boundary *Ct* value specified in the *Important Product Information Bulletin* and the *Ct* value in the Cy5 channel is less than the specified boundary *Ct* value.

The sample is considered negative for *HBV* DNA, *HCV* RNA, *HIV*-1 RNA and *HIV*-2 RNA if the *Ct* value defined in respective channel is absent or greater than the boundary *Ct* value specified in the *Important Product Information Bulletin* and *Ct* value in Cy5 channel is less than the specified boundary *Ct* value.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

When using PCR kit variant FRT-4x the channel for the Cy 5.5 fluorophore is not used and the analysis for this channel is not performed.

The results of the analysis are considered reliable only if the results for control samples are correct (see Table 5).

Table 5

Results for controls

Control	Ct in the channel for the fluorophore				
	FAM	**JOE**	**ROX**	**Cy5**	**Cy5.5**
C–	absent	absent	absent	≤ *Ct* value specified in the bulletin (positive)	absent
PCE	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)
NCA	absent	absent	absent	absent	absent
C+	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)	≤ *Ct* value specified in the bulletin (positive)

For boundary (maximum) values see *Important Product Information Bulletin*.

10. TROUBLESHOOTING

- If the *Ct* value in the channels FAM, JOE, ROX or Cy5.5 is greater than the boundary *Ct* value and the *Ct* value in the Cy5 channel is also greater than the boundary *Ct* value, PCR should be repeated for this sample starting from the RNA/DNA extraction stage.
- If the *Ct* value for the Positive Control of Extraction (PCE) or Positive Control of Amplification (C+) in any channel is absent or greater than the specified boundary *Ct* value, PCR should be repeated for all the samples starting from the RNA/DNA extraction stage.
- If the *Ct* value is present for the Negative Control of Extraction (C–) in FAM, JOE, ROX, Cy5.5 channels and/or Negative Control of Amplification (NCA) in any channel, PCR should be repeated for all positive samples starting from the RNA/DNA extraction stage.

11. TRANSPORTATION

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HCV* / *HBV* / *HIV*-FRT** PCR kit are to be stored at temperature from minus 24 to minus 16 °C when not in use. All components of the

AmpliSens® *HCV* / *HBV* / *HIV*-FRT PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV*1 / *HIV*2 and RT-PCR-mix-1-FRT *HCV* / *HBV* / *HIV* are to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® *HCV* / *HBV* / *HIV*-FRT** PCR kit is presented in the table below.

Volume of sample for extraction, µl	Method of extraction	Analytical sensitivity			
		***HCV*, IU/ml**	***HBV*, IU/ml**	***HIV*-1, copies/ml**	***HIV*-2*, copies/ml**
100	RIBO-sorb RIBO-prep NucliSENS easyMAG	100	50	200	600
200	MAGNO-sorb	50	25	100	300
1000	NucliSENS easyMAG QIAsymphony Virus/Bacteria Midi Kit	10	5	20	60

* When using PCR kit variant FRT-4x the *HIV*-2 RNA is not being detected.

The claimed analytical features of **AmpliSens® *HCV* / *HBV* / *HIV*-FRT** PCR kit are guaranteed only when additional reagents kits RIBO-sorb, RIBO-prep, MAGNO-sorb (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology"), or QIAsymphony SP or NucliSENS easyMAG automated system kit are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *HCV* / *HBV* / *HIV*-FRT** PCR kit is ensured by selection of specific primers and probes and stringent reaction conditions. The primers and probes were tested for possible homologies to all sequences deposited in gene banks by sequence comparison analysis as well as with genomic DNA/RNA of the following organisms and viruses: hepatitis A virus; hepatitis D virus; cytomegalovirus; Epstein-Barr virus; herpes simplex virus types 1 and 2; *Varicella-Zoster virus*; human herpes virus types 6 and 8; parvovirus B19; tick-borne encephalitis virus; *West Nile virus*; adenovirus types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes, S.agalactiae;*

and *Homo sapiens*. Specificity of **AmpliSens® *HCV* / *HBV* / *HIV*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines to **AmpliSens® *HCV* / *HBV* / *HIV*-FRT** PCR kit for simultaneous detection of hepatitis C virus RNA (*HCV*), hepatitis B virus DNA (*HBV*) and human immunodeficiency virus RNA (*HIV*) in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *HCV* / *HBV* / *HIV*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
RUO	Research use only		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
	Caution	**IC**	Internal control
		PCE	Positive Control of Extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
25.08.10	Page footer	Reference number is changed from R-V50-4x(RG,iQ,Mx,Dt) to R-V50-4x(RG,iQ,Mx,Dt)-CE
	3. Content	
08.07.11 LA	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that PCR-mixe-1-FRT is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
10.09.11 RT	Text	Corrections through the text
		Mention about MAGNO-sorb kit was deleted
		QIAsymphony automated system was added
		Channel for detection of *HBV* DNA was changed from ROX/TexasRed/Orange to ROX/Orange
		PCR kit variant FRT was changed into PCR kit variant FRT-4x
	Content	The volumes of Negative Control (C-) and Positive Control *HCV* / *HBV* / *HIV*-rec are changed from 1.2 ml and 0.3 ml into 0.5 ml and 0.2 ml respectively
	Sampling and handling	Procedure of preparing plasma samples was corrected
		Information about blood serum was added
	8. Protocol 8.2. Preparing the reverse transcription and PCR	The volume of added Buffer for elution was changed from 10 µl into 30 µl
	8.3. Reverse transcription and amplification	Table with amplification program for plate-type instruments was deleted
	9. Data analysis	Item was corrected (information about interpretation of results was added)
		Table “Results for controls” was changed
	10. Troubleshooting	Item was changed
	13. Specifications 13.2. Specificity	Information about genomic DNA/RNA of different organisms and viruses was added
10.07.12 IVI	Title page, Key to symbols used	Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only
17.07.13 ME	Content	The form in bulk was added
	References	The link for guidelines was added
	Page footer	**REF** R-V50-4x(RG,iQ,Mx,Dt)-CE-B was added

VER	Location of changes	Essence of changes
04.03.14 ME	8.1. RNA/DNA extraction	MAGNO-sorb kit was added. Additions and improvements of using RIBO-sorb and RIBO-prep kits were added
	8.2. Preparing reverse transcription and PCR	Table 1 was added from Appendix. The numeration of the tables was corrected
		Table 3 “Program AmpliSens *HBV* / *HCV* / *HIV* for plate-type instruments” was added
	14. References	The references were corrected
10.02.15 ME	Footer	**REF** R-V62(RG,Dt)-CE was added
	Content	PCR kit variant FRT was added
	Principle of PCR detection, Additional requirements, Protocol, Data analysis, Troubleshooting, Stability and storage, Specifications	Additions about using the PCR kit variant FRT was added