AmpliSens® *HCV*-FRT PCR kit

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by-date
RUO	Research use only		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C–	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
	Caution	PCE	Positive control of extraction
		IC	Internal control

1. INTENDED USE

AmpliSens® *HCV*-FRT PCR kit is not a medical device. PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of hepatitis C virus (*HCV*) RNA in the biological material (blood plasma) using real-time hybridization-fluorescence detection of amplified products. The material for RT-PCR is RNA samples extracted from blood plasma.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material taken in order to detect hepatitis C.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the RNA extraction from test material together with the exogenous internal control sample (Internal Control-FL (IC)), simultaneous RNA reverse transcription and amplification of cDNA fragments of the detected virus and cDNA of the Internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

RNA reverse transcription is performed with the use of TM-Revertase enzyme. Amplification of cDNA fragments is performed with the use of specific primers and Taq-polymerase enzyme.

In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. The real-time monitoring of fluorescence intensities during the real-time PCR allows detection of the amplified product without re-opening the reaction tubes after the PCR run.

AmpliSens® *HCV*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. The "hot-start" is guaranteed by the separation of nucleotides and Taq polymerase by using a chemically modified polymerase (TaqF). The latter is activated by heating at 95 °C for 15 min.

In the RT-PCR step, two target cDNAs are amplified simultaneously in the same tube.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
cDNA-target	Internal Control *ICZ*-rec (IC) cDNA	*HCV* cDNA
Target gene	Artificially synthesized sequence	5'UTR

3. CONTENT

AmpliSens® *HCV*-FRT PCR kit is produced in 2 forms:

variant FRT, REF R-V1-Mod(RG,iQ,Mx,Dt)-CE,

variant FRT in bulk[1], REF R-V1-Mod(RG,iQ,Mx,Dt)-CE-B.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.015	4 tubes
RT-PCR-mix-1-FL *HCV*	clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-buffer-C	colorless clear liquid	0.2	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	4 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.01	4 tubes
DNA calibrator PIC2 *HCV**	colorless clear liquid	0.1	4 tubes
Buffer for elution	colorless clear liquid	1.2	2 tubes
Negative Control (C–)**	colorless clear liquid	1.2	4 tubes
Positive Control-1-*HCV****	colorless clear liquid	0.06	4 tubes
Internal Control *ICZ*-rec (IC)****	colorless clear liquid	0.28	4 tubes

* serves as a Positive Control of RT-PCR (C+).

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

*** must be added during the RNA extraction procedure directly to the sample/lysis mixture.

**** must be added during the RNA extraction procedure directly to the sample/lysis mixture.

Variant FRT is intended for 112 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Vacuum tubes for sampling, storage and transportation of blood samples.
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Disposable tightly closed polypropylene 2.0-ml tubes for sampling and pretreatment.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For RNA extraction, reverse transcription and amplification

- RNA extraction kits or Automated station for RNA extraction based on magnetic beads with MAGNO-sorb Nucleic Acid Extraction kit.
- Set of consumables for the used automated station according to the manufacturer's recommendations.
- Sterile pipette tips with aerosol filters (up to 100 and up to 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); CFX 96 (Bio-Rad, USA).
- Disposable polypropylene tubes:
  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  c) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Pipettes (adjustable).
- Refrigerator at 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and laboratory coat.

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

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 (specimens, 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 afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in 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 in 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

AmpliSens® *HCV*-FRT PCR kit is intended for analysis of the RNA extracted with an RNA extraction kits from the biological material (blood plasma*)*.

Sampling

Blood plasma

Blood samples are taken after overnight fasting or in 3 hours after a meal by a disposable 0.8-1.1 mm diameter needle into a tube (special vacuum system) with 6% EDTA solution (K_2EDTA or K_3EDTA) or sodium citrate solution as an anticoagulant. Closed tubes with blood are turned several times upside down and back again to ensure that the blood in the tube is thoroughly mixed with the anticoagulant (otherwise, the blood will clot and RNA extraction will not be possible!), and stored at a temperature of 2 to 8 ° C for no more than 6 hours. Tubes with blood are centrifuged at 800–1600 g for 20 min at room temperature. The obtained plasma is transferred in an amount of at least 1 ml by separate tips with filter into sterile Eppendorf tubes with a volume of 2.0 ml.

The blood samples can be stored prior to PCR assay:

- at the temperature from 2 to 8 °C - for 3 days;
- at the temperature from minus 24 to minus 16 °C - for 1 year;
- at the temperature not more than minus 68 °C - for a long time.

Only one freeze-thaw cycle is allowed.

Blood plasma can be transported at a temperature from 2 to 8 ° C for no more than 3 days.

In some cases, blood serum can be used. The diagnostic sensitivity may be significantly decreased as a result of precipitation of viral particles during blood clot retraction. Blood serum can be stored at the temperature from 2 to 8 °C for at most 3 days or at the temperature not more than minus 68 °C for a long time.

Pretreatment

Pretreatment of blood plasma samples is not required.

Interfering substances and limitations of using test material samples

In order to control the RNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control *ICZ*-rec (IC)) is used in the PCR kit. The Internal Control is added to each biological sample at the extraction stage. After the amplification the presence of the signal, indicating the accumulation of cDNA IC *ICZ*-rec fragments, testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

Limitations of using test material samples

The next samples are inapplicable for analysis:

- blood plasma samples with hemolysis;
- the blood plasma samples, which has been exposed to repeated freezing-thawing.
- the blood plasma samples, collected in the tubes with heparin as anticoagulant;

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material (blood plasma) used for the study were selected to assess potential interference.

The following were tested:

- *HCV*-negative blood plasma samples without and with the addition of endogenous potentially interfering substances. The concentration of each potentially interfering substance is given in Table 2. Both samples without *HCV* RNA and samples with added quality control sample (QCS) up to a final *HCV* RNA concentration of 500 IU/mL were analyzed;
- blood plasma samples obtained from whole blood drawn in tubes with K_2EDTA and K_3EDTA as anticoagulants.

Table 2

Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Endogenous substances	Hemoglobin	160 g/l (upper limit of normal)	Not detected
		250 g/l	
	Tryglicerides	3.7 mmol/l (upper limit of normal)	
		37.0 mmol/l	
	Bilirubin	21 µmol/l (upper limit of normal)	
		210 µmol/l	
	Protein	85 g/l (upper limit of normal)	
		120 g/l	
Exogenous substances	Lithium heparin	12 IU/ml	Detected
	Potassium EDTA	2.0 mg/ml	Not detected

7. WORKING CONDITIONS

AmpliSens® *HCV*-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. RNA extraction

NOTE: Only sterile disposable plastic consumables with special RNase-free, DNase-free markings should be used for work with RNA.

It is recommended that the following nucleic acid extraction kits are used:

- **RIBO-sorb**;
- **RIBO-prep**;
- **MAGNO-sorb**.

The RNA extraction of each test and control sample is carried out in the presence of **Internal Control *ICZ*-rec (IC)**.

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

- Add **10 µl** of **Internal Control *ICZ*-rec (IC)** to each tube and then add **450 µl** of **Lysis Solution**.
- For a large number of samples, to facilitate the extraction procedure, it is allowed to mix **Lysis Solution** and **Internal Control *ICZ*-rec (IC)** (450 µl **Lysis Solution** and 10 µl **Internal Control ICZ-rec (IC)** per sample) and then add **450** µl of mixture into each prepared 1.5-ml tubes.
- It is necessary to carry out the positive and negative controls of extraction. To the tube labelled PCE add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HCV***. To the tube labelled C– add **100 µl** of **Negative Control (C–).** Close the tubes. Vortex thoroughly and sediment the drops from the caps of the tubes by short centrifugation
- After addition of biological and control samples to **Lysis Solution** warm the mixture at 60 °C for 10 min prior to sorbent addition. Sediment the drops from the caps of the tubes by short centrifugation (perform this step only in case simultaneous extraction *HCV* RNA and *HBV* DNA and/or *HDV* RNA)

NOTE: **If using RIBO-prep kit**, extract the RNA according to the manufacturer's protocol:

The volumes of reagents and samples when the RNA is extracted by the RIBO-prep reagent kit:

- Add **10 µl** of the **Internal Control *ICZ*-rec (IC)** into each tube
- The volume of the test sample is **100 µl**
- Add **100 µl** of **Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction)
- Add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HCV*** to the tube labelled PCE (Positive Control of Extraction).
- The volume of elution is **50 µl.**

NOTE: **If using the MAGNO-sorb kit** extract the RNA according to the manufacturer's protocol

MAGNO-sorb reagent kit can be used in combination with "open type" automatic nucleic acid extraction stations.

The volumes of reagents and samples when the RNA is extracted by the MAGNO-sorb reagent kit:

- Add **10 µl** of the **Internal Control *ICZ*-rec (IC)** into each tube.
- The volume of the test sample:
  - 200 µl;
  - 1000 µl.
- Add **100 µl** of **Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).
- Add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HCV*** to the tube labelled PCE (Positive Control of Extraction).
- The volume of elution is **70 µl** (or **75 µl** in case of extraction using MicroLab STARlet (Hamilton Bonaduz AG) Switzerland) station).

NOTE: It is allowed to store the RNA samples at the temperature from 2 to 8 °C for 4 hours, at the temperature from minus 24 to minus 16 °C for 1 month and at the temperature not more than minus 68 °C for 1 year.

8.2. Preparing reverse transcription and PCR

8.2.1 Preparing tubes for RT-PCR

The total reaction volume is **25 µl**, the volume of the **RNA** is **10 µl**.

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, RNA and control samples into tubes.

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

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   - **10 µl** of **RT-PCR-mix-1-FL *HCV***,
   - **5 µl** of **PCR-buffer-C**,
   - **0.25 µl** of **RT-G-mix-2,**
   - **0.5 µl** of **Polymerase (TaqF),**
   - **0.25 µl** of **TM-Revertase (MMlv)**.

Prepare the reaction mixture for the total number of test and control samples plus some extra reaction. See numbers of control samples in item 7.

The calculation for the required number of reactions can be performed according to Table 3.

Table 3

Scheme of reaction mixture preparation

		Reaction volume (with allowance for one extra sample)				
Reagent volume for one reaction, µl		10.00	5.00	0.25	0.50	0.25
Number of test samples	Number of PCR reactions[2]	RT-PCR-mix-1-FL *HCV*	PCR-buffer-C	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMlv)
4	**7**	**80**	**40**	**2.0**	**4.0**	**2.0**
6	**9**	**100**	**50**	**2.5**	**5.0**	**2.5**
8	**11**	**120**	**60**	**3.0**	**6.0**	**3.0**
10[3]	**13**	**140**	**70**	**3.5**	**7.0**	**3.5**
12	**15**	**160**	**80**	**4.0**	**8.0**	**4.0**
14	**17**	**180**	**90**	**4.5**	**9.0**	**4.5**
16	**19**	**200**	**100**	**5.0**	**10.0**	**5.0**
18	**21**	**220**	**110**	**5.5**	**11.0**	**5.5**
20	**23**	**240**	**120**	**6.0**	**12.0**	**6.0**
22[4]	**25**	**260**	**130**	**6.5**	**13.0**	**6.5**
34	**37**	**380**	**190**	**9.5**	**19.0**	**9.5**
46	**49**	**500**	**250**	**12.5**	**25.0**	**12.5**

[2] Number of biological samples + 2 controls of RNA extraction + 1 control of RT-PCR (N+3, N - number of biological samples).
[3] 12-tube panel for extraction.
[4] 24-tube panel for extraction.

NOTE: Prepare the reaction mixture just before use.

2 Thaw all the tubes of PCR kit, thoroughly vortex them and sediment the drops by short centrifugation.

3 In a new tube prepare the reaction mixture. Mix the required quantities of **RT-PCR-mix-1-FL *HCV*, PCR-buffer-C, RT-G-mix-2, Polymerase (TaqF)** and **TM-Revertase (MMlv)**. Vortex thoroughly and sediment the drops from the caps of the tubes by short centrifugation. Do not store the prepared mixture.

4 Take the required number of the tubes for RT-PCR of test and control samples (see Table 3).

5 Add **15 µl** of the prepared reaction mixture to each PCR tube.

6 Add **10 µl** of **RNA samples** extracted from the biological samples to each PCR tube.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

NOTE: Avoid transferring sorbent together with the RNA sample in case of extraction using the reagent kit on silica gel or magnetic separation.

7 Carry out the **control reactions**:

PCE – Add **10 µl** of the **RNA sample** extracted from the Positive Control-1-*HCV* to the tube labeled PCE (Positive Control of Extraction)

C– – Add **10 µl** of the **RNA sample** extracted from the Negative Control to the tube labeled C– (Negative Control of Extraction)

C+ – Add **10 µl** of **DNA calibrator PIC2 *HCV*** to the tube labeled C+ (Positive Control of RT-PCR).

To rule out possible contamination, carry out an additional control reaction:

NCA – Add **10 µl** of **Buffer for elution** to the tube labeled NCA (Negative Control of RT-PCR).

8.2.2. Reverse transcription and amplification

1. Create a temperature profile on your instrument as follows:

Table 4

AmpliSens-2 RG program for rotor-type instruments[5]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
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	
	72	15 s	–	

Table 5

AmpliSens-2 iQ program for plate-type instruments[6]

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	
	72	15 s	–	

NOTE: Any combination of the tests (for example simultaneously with *HDV* detection tests; *HCV* genotyping and etc.) can be performed in one instrument simultaneously with the use of either AmpliSens-2 RG or AmpliSens-2 iQ programs.

NOTE: Channels ROX and Cy5 are switched on when necessary

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

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

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

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

Table 6

Channel for the fluorophore	FAM	JOE
Amplification product	Internal Control *ICZ*-rec (IC) cDNA	*HCV* cDNA

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the RNA sample in the corresponding column of the results grid.
Principle of interpretation is the following:

Table 7

Results interpretation for the test samples

Ct value in the channel for the fluorophore		Result
FAM	JOE	
< boundary value	absent or > boundary value	***HCV* cDNA is NOT detected**
> or < boundary value	< boundary value	***HCV* cDNA is detected**
absent or > boundary value	absent or > boundary value	**Invalid***

* In case of **invalid** result, the PCR analysis should be repeated starting from the RNA extraction stage for the corresponding test sample.

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

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Positive and Negative Control of extraction are correct (see Table 8).

Table 8

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	RNA extraction	<boundary value	Absent
PCE	RNA extraction	<boundary value	<boundary value
NCA	RT-PCR	Absent	Absent
C+	RT-PCR	<boundary value	<boundary value

Interpretation of some test samples is not possible if the results for the controls deviate from the results specified above (see *10.Troubleshooting*).

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. For the Positive Control of Extraction (PCE) and/or Positive Control of RT-PCR (C+):
   a) The *Ct* value in the channel for the JOE fluorophore is absent or exceeds the boundary value. It is not possible to interpret the results for samples in which *HCV* cDNA is not detected. It is necessary to repeat the PCR analysis for all samples in which *HCV* cDNA is not detected, starting from the RNA extraction stage.
   b) The *Ct* value in the channel for the FAM fluorophore is absent or exceeds the boundary value. It is impossible to interpret the results for the test samples. It is necessary to repeat the PCR analysis for all samples starting from the RNA extraction stage.
2. For the Negative Control of Extraction (C–):
   a) The *Ct* value is determined in the channel for the JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents / test samples is probable at any stage of PCR analysis. Results cannot be interpreted for samples in which *HCV* cDNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which *HCV* cDNA is detected.
   b) The *Ct* value in the channel for the FAM fluorophore is absent or exceeds the boundary value. It is not possible to interpret the results for the tested samples. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples.
3. The *Ct* value is determined for the Negative Control of RT-PCR (NCA) in the channels for FAM and/or JOE fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents / test samples is probable at any stage of PCR analysis. Results cannot be interpreted for samples in which *HCV* cDNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples.

11. TRANSPORTATION

AmpliSens® *HCV*-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*-FRT** PCR kit are to be stored at temperature from minus 24 to minus 16 °C when not in use. They are stable until the expiration date indicated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: DNA calibrator PIC2 *HCV*, Positive Control-1-*HCV,* and Internal Control *ICZ*-rec (IC) should not be frozen/thawed more than twice. After thawing, PIC2 *HCV*, Positive Control-1-*HCV,* and Internal Control *ICZ*-rec (IC) should be stored at 2-8 °C for at most 6 months.

NOTE: RT-PCR-mix-1-FL *HCV* is to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 9

Biological material	Volume of sample for extraction, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity (limit of detection), UI/ml
Blood plasma	100	RIBO-sorb	PCR kit variant FRT	100
		RIBO-prep		
	200	MAGNO-sorb		50
	1000	MAGNO-sorb		10

The claimed features are achieved while respecting the rules specified in the section "*Sampling and Handling".*

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *HCV*-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.
The reagent kit detects *HCV* RNA of different genotypes. Samples of genotypes 1a, 2b, 3a, 4a, 5, 6a at a concentration of at least $1x10^3$ IU/ml were tested.
Analytical specificity of the reagent kit was proved by adding genomic DNA/RNA of the following microorganisms and viruses at a concentration of at least $1x10^5$ copies/ml (GE/ml): hepatitis A virus (*HAV*); hepatitis B virus (*HBV*); hepatitis D virus (*HDV*); human immunodeficiency virus (*HIV*); cytomegalovirus (*CMV*); Epstein-Barr virus (EBV); herpes simplex virus types 1 and 2 (*HSV*-1,2); *varicella-zoster* virus (*VZV*); human herpes virus types 6 and 8 (*HHV*-6, *HHV*-8); *Parvovirus* B19; tick-borne encephalitis virus (*TBEV*); West Nile encephalitis (*WNV*); adenovirus types 2, 3, and 7 (*HadV*-2, *HadV*-3, *HadV*-7); human papillomavirus types 6, 11, 16, 18, 31, 33, 35, 39, 45, 59, *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes, Streptococcus agalactiae;* as well as human DNA at a concentration of 1 mg/ml.
There were no nonspecific responses in tests with RNA/DNA of the above-mentioned microorganisms/viruses, as well as human DNA.
The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

[5] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).
[6] For example, CFX 96 (Bio-Rad, USA).

13.3. Reproducibility and repeatability

Repeatability and reproducibility were determined by testing positive and negative model samples. Positive samples were dilutions of quality control sample (QCS) containing *HCV* RNA with a concentration of 500 UI/ml. Negative control (C–) reagent was used as a negative sample.

Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing in two independent laboratories, by different operators, on different days, on different equipment and different reagent kit series. The results are presented in Table 10.

Table 10

Sample type	Repeatability		Reproducibility	
	Number of samples	Agreement of results, %	Number of samples	Agreement of results, %
Positive	10	100	40	100
Negative	10	100	40	100

14. REFERENCES

1. Andrade E., Daniele Rocha D., Fontana-Maurell M., et al. One-step real-time PCR assay for detection and quantification of RNA HCV to monitor patients under treatment in Brazil // Braz J Infect Dis. 2018 Sep-Oct;22(5):418-423. doi: 10.1016/j.bjid.2018.08.003.
2. Da Silva M.C.C., DE Abreu L.C.L., DO Carmo F.A., et al. Development of a validation protocol method for nucleic acid testing to detect human immunodeficiency virus, hepatitis C virus, and hepatitis B virus // An Acad Bras Cienc. 2022 Nov 21;94(suppl 3):e20211321. doi: 10.1590/0001-3765202220211321.
3. Hongjaisee S., Jabjainai Y., Sakset S., et al. Comparison of Simple RNA Extraction Methods for Molecular Diagnosis of Hepatitis C Virus in Plasma. Diagnostics 2022, 12, 1599. https://doi.org/10.3390/ diagnostics12071599.

15. QUALITY CONTROL

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

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.06.10	Page footer	Reference number is changed from R-V1-Mod(RG,iQ,Mx,Dt)-E to R-V1-Mod(RG,iQ,Mx,Dt)-CE
	Content, text	Name of Positive Control of amplification is changed from KB2 to PIC2
04.12.10	Sampling and handling	Sentence «Blood samples are taken after overnight fasting into tubes with 3% EDTA solution (1 : 20)» is changed into «Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant».
	Data analysis	Mention about DT-96 device is deleted
	Through the text	MAGNO-sorb mention is deleted
		Corrections through the text
13.12.10	Through the text	Name of Positive Control of amplification is changed from Positive Control PIC2 *HCV* (C+) to DNA calibrator PIC2 *HCV*
05.07.11 LA	Cover page	The phrase "For Professional Use Only" 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 RT-PCR-mix-1-FL *HCV* 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"
15.09.11 RT	8. PROTOCOL 8.1. RNA/DNA extraction	The information about using RIBO-prep kit was added
20.06.12 LA	Cover page	Symbol IVD was replaced by RUO symbol
	16. Key to symbols used	
	13.1. Sensitivity	Information related to MAGNO-sorb reagent kit was added to the table of analytical sensitivity
	8.1. DNA extraction	Reference number of MAGNO-sorb reagent kit was added: K2-16-1000-CE
		Information about extraction with MAGNO-sorb is added
04.02.14 ME	8.1. RNA extraction	The information about using EM-plus reagent kit was deleted. The chapter was rewritten
	8.2. Preparing the PCR	Table 1 was added from Appendix. The tables through the text was numerated
	10. Data analysis	The chapter was rewritten
	11. Troubleshooting	The chapter was corrected
	14. References	The references was corrected
	16. Key to symbols used	The explanation of PCE was added
	8.1. RNA extraction	Information about preparing the controls of extraction was added
	13.2. Specificity	The phrase "The clinical specificity of AmpliSens® *HCV*-FRT PCR kit was confirmed in laboratory clinical trials" was deleted
03.04.15 ME	1. Intended use	The phrase "The results of PCR analysis are taken into account in complex diagnostics of disease" was changed to "For research use only. Not for diagnostic procedures". Clinical material was changed to biological
16.06.17 ME	8.1. RNA extraction	Information about preparing the controls of extraction was added
30.08.18 EM	13.2. Specificity	The phrase "The clinical specificity of AmpliSens® *HCV*-FRT PCR kit was confirmed in laboratory clinical trials" was deleted
21.07.20	Through the text	The text formatting was changed
KK	Footer	The phrase "For research use only. Not for diagnostic procedures" was added
12.08.20 MM	2. Principle of PCR detection	The table with targets was added
02.11.20 MM	2. Principle of PCR detection	Corrections according to the template
16.11.20 KK	Footer	The REF R-V1-Mod(RG,iQ,Mx,Dt)-CE-B was deleted
	3. Content	The form in bulk was deleted
01.06.21 MM	Footer	The REF R-V1-Mod(RG,iQ,Mx,Dt)-CE-B was added
	3. Content	The form in bulk was added
01.02.24 BA	Key to symbols used	The explanation of PCE was changed from "Positive control of Amplification" to "Positive control of extraction"
	Through the text	Corrections according to the template
		Name of RT-PCR-mix-2-FEP/FRT is changed to PCR-buffer-C
	4. Additional requirements	The section was actualized and updated with materials and instruments. Automated station for RNA extraction, Rotor-Gene Q and CFX 96 were added. Mx3000P and iCycler iQ5 were deleted
	6. Sampling and handling	The information about sampling and handling was expanded. The subsection *Interfering substances and limitations of using test material samples* was added
	8. Protocol	NucliSENS easyMAG automated was deleted The section was updated with the information about extraction using RIBO-sorb, RIBO-prep and MAGNO-sorb extraction kits.
	9. Data analysis	Information on the correspondence of the amplification product and channels for the fluorophore, the principle of results interpretation for the test samples and controls are presented in tables
	10. Troubleshooting	The section was rewritten
	13. Specifications	The "13.1. Analytical sensitivity (limit of detection)" and "13.2. Analytical specificity" subsections were actualized. The "13.3. Reproducibility and repeatability" subsection was added
	14. References	The section was actualized
07.06.24 PM	1. Intended use	The information about indications and contra-indications for use of the reagent kit was added

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