

**RUO**

For Professional Use Only

# **AmpliSens<sup>®</sup> HCV-FRT**

PCR kit

## **Instruction Manual**

**AmpliSens<sup>®</sup>**



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## 1. INTENDED USE

**AmpliSens® HCV-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of hepatitis C virus (*HCV*) RNA in the biological materials (blood plasma) using real-time hybridization-fluorescence detection.



For research use only. Not for diagnostic procedures

## 2. PRINCIPLE OF PCR DETECTION

Hepatitis C virus detection includes (1) RNA/DNA extraction from blood plasma together with the internal control sample (IC), which must be used in the extraction procedure to control the extraction of each individual sample and to detect possible reaction inhibition, (2) reverse transcription of RNA, and (3) real-time PCR amplification of cDNA. *HCV* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *HCV* primers. 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 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.

The IC amplification product is detected in the channel for the FAM fluorophore. The *HCV* cDNA amplification product is detected in the channel for the JOE fluorophore. The Positive Control of Extraction, **Positive Control-1-HCV**, is detected in the channels for the FAM (IC) and JOE (*HCV*) fluorophores. The Positive Control of Amplification, **DNA calibrator PIC2 HCV**, is a complex control for *HCV* and IC. It is detected in the channels for the FAM (IC) and JOE (*HCV*) fluorophores.

## 3. CONTENT

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

AmpliSens® *HCV-FRT* PCR kit variant FRT, **REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE.

AmpliSens® *HCV-FRT* PCR kit variant FRT in bulk<sup>1</sup>, **REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE-B.

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<sup>1</sup> In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

AmpliSens® HCV-FRT PCR kit includes:

| <b>Reagent</b>                           | <b>Description</b>      | <b>Volume, ml</b> | <b>Quantity</b> |
|--|-------------------------|-------------------|-----------------|
| <b>RT-G-mix-2</b>                        | colorless, clear liquid | 0.015             | 4 tubes         |
| <b>RT-PCR-mix-1-FL HCV</b>               | colorless, clear liquid | 0.3               | 4 tubes         |
| <b>RT-PCR-mix-2-FEP/FRT</b>              | colorless, clear liquid | 0.2               | 4 tubes         |
| <b>Polymerase (TaqF)</b>                 | colorless, clear liquid | 0.02              | 4 tubes         |
| <b>TM-Revertase (MMIv)</b>               | colorless, clear liquid | 0.01              | 4 tubes         |
| <b>DNA calibrator PIC2 HCV*</b>          | colorless, clear liquid | 0.1               | 4 tubes         |
| <b>Buffer for elution</b>                | colorless, clear liquid | 1.2               | 2 tubes         |
| <b>Negative Control (C-)**</b>           | colorless, clear liquid | 1.2               | 4 tubes         |
| <b>Positive Control-1-HCV***</b>         | colorless, clear liquid | 0.06              | 4 tubes         |
| <b>Internal Control ICZ-rec (IC)****</b> | colorless, clear liquid | 0.28              | 4 tubes         |

\* Serves as a Positive Control of Amplification (C+).

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

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

\*\*\*\* Must be added during the RNA/DNA extraction procedure directly to the sample/lysis mixture.

AmpliSens® HCV-FRT PCR kit is intended for 112 amplification reactions including controls.

#### 4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit or RNA/DNA extraction automatic station.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase/DNase-free pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA)).

- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  - a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  - b) 0.2-ml PCR tubes with flat caps PCR tubes if a rotor-type instrument is used.
- Refrigerator at 2 to 8 °C.
- Deep-freezer at 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 is described in detail in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting the work.

**AmpliSens<sup>®</sup> HCV-FRT** PCR kit is intended for analysis of RNA extracted with an RNA/DNA extraction kits from

- *Peripheral blood plasma.*

Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant. Closed tubes with blood are turned several times upside down and back again. Blood plasma should be taken and transferred to new tubes within 6 h after taking blood. For this purpose, tubes with blood are centrifuged at 800–1600 g for 20 min. Blood plasma can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at or below 68 °C) for a long time.

In some cases, blood serum can be used. In this case, the analytical sensitivity of the reagent kit is retained; however, the clinical sensitivity may be significantly decreased as a result of precipitation of viral particles during blood clot retraction. Blood serum can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at or below 68 °C) for a long time.

## 7. WORKING CONDITIONS

**AmpliSens<sup>®</sup> HCV-FRT** PCR kit should be used at 18–25 °C.

## 8. PROTOCOL

### 8.1. RNA extraction

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

- RIBO-sorb, **REF** K2-1-Et-100-CE ;
- RIBO-prep, **REF** K2-9-Et-100-CE ;
- MAGNO-sorb, **REF** K2-16-1000-CE;
- NucliSENS easyMAG automated nucleic acid extraction system (bioMérieux, France) can also be used.



**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 ICZ-rec** to each tube and then add **450 µl** of **Lysis Solution**.

**REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE; **REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE-B /

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- 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 (as its shown in RIBO-sorb instruction manual).
- For each panel 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–)**.
- After addition of biological and control samples to lysis solution warm the mixture at 60 °C for 10 min prior to sorbent addition.



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

- 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 (as its shown in RIBO-prep instruction manual).
- For each panel 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–)**.



**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 extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control ICZ-rec** required for 24-tube panel is **0.28 ml**. In case of other panels and RNA extraction from blood plasma sample of 200 µl see MAGNO-sorb instruction manual.
- 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 (as its shown in MAGNO-sorb instruction manual).
- For each panel 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–)**.
- The volume of **Buffer for elution** required for extraction from both 1000 and 200 µl of blood plasma samples is **70 µl**.



**If using NucliSENS easyMAG automated system:**

- Use protocols and reagents allowed carrying out RNA/DNA extraction from blood plasma and serum in volume from 0.1 to 1 ml.
- **Internal Control ICZ-rec** (10 µl per sample) addition to the samples or lysis solution before beginning of the extraction is required.
- 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).
- For each panel 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–)**.
- Set the eluate volume as 50-60 µl (up to 100 µl).

**REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE; **REF** R-V1-Mod(RG,iQ,Mx,Dt)-CE-B /

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- Both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation modes can be used.
- Then the RNA extraction is completed, take the tubes from the device and carry out the RT-PCR reaction. Purified RNA can be stored at 2–8 °C for 4 hours, at not more than minus 16 °C for one month and at not more than minus 68 °C for one year.

For details, see the Guidelines [2].

## 8.2. Preparing the reverse transcription and PCR

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



All components of the reaction mixture should be mixed immediately before use. Mix reagents for the required number of reactions for experimental and control samples according to table 1.

### 8.2.1 Preparing tubes for PCR

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Take the required number of tubes for amplification of biological and control samples (including two controls of extraction and one control of amplification). The type of tubes depends on the real-time PCR instrument used for analysis.
3. **To prepare the reaction mixture**, mix the reagents (**10 µl of RT-PCR-mix-1-FL HCV**, **5 µl of RT-PCR-mix-2-FEP/FRT**, **0.25 µl of RT-G-mix-2**, **0.5 µl of Polymerase (TaqF)**, and **0.25 µl of TM-Revertase (MMIv)** per one reaction) in a new sterile tube (see also table 1). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 1

### Scheme of reaction mixture preparation

| Reagent volume for one reaction, µl |                                      | Reaction volume (with allowance for one extra sample) |                      |            |                   |                     |
|-------------------------------------|--------------------------------------|---|----------------------|------------|-------------------|---------------------|
|                                     |                                      | 10.00   | 5.00                 | 0.25       | 0.50              | 0.25                |
| Number of biological samples        | Number of PCR reactions <sup>1</sup> | RT-PCR-mix-1-FL HCV                                   | RT-PCR-mix-2-FEP/FRT | RT-G-mix-2 | Polymerase (TaqF) | TM-Revertase (MMIv) |
| 4                                   | 7                                    | 80  | 40                   | 2.0        | 4.0               | 2.0                 |
| 6 <sup>2</sup>                      | 9                                    | 100   | 50                   | 2.5        | 5.0               | 2.5                 |
| 8                                   | 11                                   | 120   | 60                   | 3.0        | 6.0               | 3.0                 |
| 10 <sup>3</sup>                     | 13                                   | 140   | 70                   | 3.5        | 7.0               | 3.5                 |
| 12                                  | 15                                   | 160   | 80                   | 4.0        | 8.0               | 4.0                 |
| 14 <sup>4</sup>                     | 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                 |

<sup>1</sup> Number of biological samples + 2 controls of extraction + 1 control of PCR (N+3, N - number of biological samples).

<sup>2</sup> Extraction in one stripe in NucliSENS easyMAG (8 tubes).

<sup>3</sup> 12-tube panel for extraction.

<sup>4</sup> Extraction in two stripes in NucliSENS easyMAG (16 tubes).



|                 |    |     |     |      |      |      |
|-----------------|----|-----|-----|------|------|------|
| 22 <sup>5</sup> | 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 |

- Add **15 µl** of the prepared reaction mixture to each PCR tube.
- Add **10 µl** of **RNA samples** extracted from the biological samples to each PCR tube.



Avoid transferring sorbent together with the RNA sample in case of extraction using RIBO-sorb kit, MAGNO-sorb or the NucliSENS easyMAG automated system.

- Run 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 Amplification).

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

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

## 8.2.2 Reverse transcription and amplification

- Create a temperature profile on your instrument as follows:

Table 2

### AmpliSens-2 RG program for rotor-type instruments<sup>6</sup>

| Step          | Temperature, °C | Time   | Fluorescence detection | Cycles |
|---------------|-----------------|--------|------------------------|--------|
| 1 (Hold)      | 50              | 15 min | –                      | 1      |
| 2 (Hold)      | 95              | 15 min | –                      | 1      |
| 3 (Cycling 1) | 95              | 5 s    | –                      | 5      |
|               | 60              | 20 s   | –                      |        |
|               | 72              | 15 s   | –                      |        |
| 4 (Cycling 2) | 95              | 5 s    | –                      | 40     |
|               | 60              | 20 s   | FAM, JOE, ROX, Cy5     |        |
|               | 72              | 15 s   | –                      |        |

- Adjust the fluorescence channel sensitivity as described in the Guidelines.



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).

<sup>5</sup> 24-tube panel for extraction, extraction in three stripes in NucliSENS easyMAG.

<sup>6</sup> For example, Rotor-Gene 3000 or 6000 (Corbett Research, Australia)



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

AmpliSens-2 iQ program for plate-type instruments<sup>7</sup>

| Step | Temperature, °C | Step duration | Fluorescence detection | Cycle repeats |
|------|-----------------|---------------|------------------------|---------------|
| 1    | 50              | 15 min        | –                      | 1             |
| 2    | 95              | 15 min        | –                      | 1             |
| 3    | 95              | 5 s           | –                      | 5             |
|      | 60              | 20 c          | –                      |               |
|      | 72              | 15 s          | –                      |               |
| 4    | 95              | 5 s           | –                      | 40            |
|      | 60              | 30 s          | FAM, JOE, ROX, Cy5     |               |
|      | 72              | 15 s          | –                      |               |



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).



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

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.
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:

- The signal of the Internal Control cDNA amplification product is detected in the channel for the FAM fluorophore,
- The signal of the HCV cDNA amplification product is detected in the channel for the JOE fluorophore.

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:

<sup>7</sup> For example, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent

- The sample is considered **positive** for *HCV* RNA if the *Ct* value detected in the channel for the JOE fluorophore does not exceed the boundary value specified in the *Important Product Information Bulletin*.
- The sample is considered **negative** for *HCV* RNA if the *Ct* value in the channel for the JOE fluorophore is absent or if the *Ct* value detected in the channel for the JOE fluorophore is greater than the specified boundary value and the *Ct* value in the channel for the FAM fluorophore does not exceed the boundary value specified in the *Important Product Information Bulletin*.
- The sample is considered **equivocal** if an equivocal result is obtained in any of the channels. In this case, PCR analysis of this sample should be repeated once again.



Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2].

**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 4).**

Table 4

**Results for controls**

| Control    | Stage for control | Ct value in the channel for fluorophore |                 |
|------------|-------------------|---|-----------------|
|            |                   | FAM                                     | JOE             |
| <b>C–</b>  | RNA extraction    | ≤boundary value                         | Absent          |
| <b>PCE</b> | RNA extraction    | ≤boundary value                         | ≤boundary value |
| <b>NCA</b> | Amplification     | Absent                                  | Absent          |
| <b>C+</b>  | Amplification     | ≤boundary value                         | ≤boundary value |

## 10. TROUBLESHOOTING

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

1. If the *Ct* value for PCE or C+ in the channel for the JOE fluorophore is absent or exceeds the specified boundary value, analysis of all samples in which *HCV* RNA was not detected should be repeated once again starting from the RNA extraction stage.
2. If the *Ct* value is determined for NCA and/or C– in the channel for the JOE fluorophore, analysis of all samples in which *HCV* RNA was detected should be repeated once again starting from the RNA extraction stage.

## 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.



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



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

## 13. SPECIFICATIONS

### 13.1. Sensitivity

The analytical sensitivity of **AmpliSens® HCV-FRT** PCR kit is specified in the table below.

| Volume of sample for extraction, µl | RNA/DNA extraction kit                      | Analytical sensitivity, IU/ml |
|-------------------------------------|---|-------------------------------|
| 100                                 | RIBO-sorb<br>RIBO-prep<br>NucliSENS easyMAG | 100                           |
| 200                                 | MAGNO-sorb                                  | 50                            |
| 1000                                | MAGNO-sorb<br>NucliSENS easyMAG             | 10                            |



The claimed analytical features of **AmpliSens® HCV-FRT** PCR kit are guaranteed only when additional reagents kits RIBO-sorb, RIBO-prep, or MAGNO-sorb (manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”) are used.

### 13.2. 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 as well as with genomic DNA/RNA of the following organisms and viruses: hepatitis A virus; hepatitis B virus; hepatitis D virus; human immunodeficiency 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 encephalitis; adenovirus types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus*; *Streptococcus pyogenes*, *S.agalactiae*; and *Homo sapiens*. Cross-reactions for the above-mentioned organisms and viruses have not been detected.













## 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-FRT*, AmpliSens® *HDV-FRT*, AmpliSens® *HBV-FRT*, AmpliSens® *HGV-FRT* PCR kits developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

## 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.

## 16. KEY TO SYMBOLS USED

|   |                        |  |                                   |
|---|------------------------|--|-----------------------------------|
|    | Catalogue number       |  | Sufficient for                    |
|    | Batch code             |   | Expiration Date                   |
|    | Research use only      |  | Consult instructions for use      |
|    | Version                |  | Keep away from sunlight           |
|    | Temperature limitation | <b>NCA</b>   | Negative control of amplification |
|    | Manufacturer           | <b>C-</b>  | Negative control of extraction    |
|   | Date of manufacture    | <b>C+</b>  | Positive control of amplification |
|  | Caution                | <b>PCE</b>   | Positive control of extraction    |
|   |                        | <b>IC</b>  | Internal control                  |

## 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<br>Corrections through the text   |
| 13.12.10         | Through the text   | Name of Positive Control of amplification is changed from Positive Control PIC2 <i>HCV</i> (C+) to DNA calibrator PIC2 <i>HCV</i>   |
| 05.07.11<br>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 <i>HCV</i> 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<br>RT   | 8. PROTOCOL<br>8.1. RNA/DNA extraction   | The information about using RIBO-prep kit was added   |
| 20.06.12<br>LA   | Cover page   | Symbol <span style="border: 1px solid black; padding: 0 2px;">IVD</span> was replaced by <span style="border: 1px solid black; padding: 0 2px;">RUO</span> 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<br>Information about extraction with MAGNO-sorb is added  |
|                  |  |   |
| 04.02.14<br>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 in accordance with Russian instruction  |
|                  | 14. References   | The references was corrected  |
|                  | 16. Key to symbols used  | The explanation of PCE was added  |



| <b>VER</b>     | <b>Location of changes</b> | <b>Essence of changes</b>   |
|----------------|----------------------------|---|
| 03.04.15<br>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”.<br>Clinical material was changed to biological |
|                | 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   |
| 16.06.17<br>ME | Footer                     | <b>REF</b> R-V1-Mod(RG,iQ,Mx,Dt)-CE-B was added   |